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STUDIES ON MALTING QUALITY

I. 1935 VARIETY TRIALS

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Information on the malting quality of barley varieties grown in Canada is required in order: (a) that sound recommendations may be made with respect to the varieties which should be grown in malting areas; (b) that plant breeders, who are striving to produce varieties equal in malting quality to O.A.C. 21 and superior to it in agronomic characters and disease resistance, may be able to select suitable parent varieties for their purposes; and (c) to indicate the varieties which for purposes of commercial grading may be considered equal in malting quality to the standard variety O.A.C. 21. This paper reports the results of malting tests, made in 1936 at the University of Manitoba, on eight smooth-awned and six rough-awned barley varieties grown at a number of experimental stations in Canada during 1935.

The laboratory malting test measures the response of samples to a standard set of malting conditions which simulate the average conditions used in commercial practice in Canada. It follows that not all samples are malted to the best advantage. There is some reason to suppose, however, that when laboratory malting tests show that two varieties differ widely in malting quality, the difference is characteristic of the varieties and would persist, though possibly to a lesser extent, if each variety were malted to the best advantage. In addition, it seems probable that varieties which modify readily and can be malted to good advantage in the laboratory are better suited to the short (6-day) germination period used in commercial practice in Canada than varieties which react unfavourably under laboratory test conditions.

While the laboratory malting test compares samples with respect to certain quantitative malting characteristics, it fails to elucidate many other important factors. For this reason, malting data must be interpreted conservatively and more weight can be placed on negative than on positive conclusions. Thus, if tests show that a variety is lacking in certain important malting qualities, then it may be assumed that it is inferior to the standard variety. On the other hand, if tests show a variety to be equal to a standard variety with respect to all factors measured, it would be unwise to assume that the variety is equal in malting quality to the standard since it may be lacking in some quality not measured in the

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test. In spite of these limitations, the laboratory malting test must be considered as a valuable means of studying barley varieties, for which there is at present no adequate substitute.

MATERIALS

The varieties which were studied and the stations at which they were grown are listed below. The numbers after each variety represent the stations from which samples of the variety were received.

Varieties

Smooth-awned
- Brandon 1099, (1-6)
- Byng, (6-13)
- Newal, (1-6)
- Nobarb, (1-13)
- Regal, (1-13)
- Velvet, (1-13)
- Wisconsin 38, (1-13)
- York, (6-13)

Rough-awned
- O.A.C. 21, (1-13)
- Olli, (3-11)
- Peatland, (3-11)
- Pontiac, (6-11)
- *Victory, (3-6)
- *Washington, (3-11)

Stations

1. University of Alberta, Edmonton, Alberta.
2. University of Manitoba, Winnipeg, Manitoba.

Dominion Experimental Farms at:—

3. Scott, Saskatchewan.
4. Indian Head, Saskatchewan.
5. Brandon, Manitoba.
6. Ottawa, Ontario.
7. Ste. Anne de la Pocatière, Quebec.
8. Lennoxville, Quebec.
9. Fredericton, New Brunswick.
10. Charlottetown, Prince Edward Island.
11. Nappan, Nova Scotia.

12. Macdonald College, Ste. Anne de Bellevue, Quebec.
13. Ontario Agricultural College, Guelph, Ontario.

The samples listed above represent part of the material studied in 1935 in the co-operative variety trials carried out by the various Agricultural Colleges and Dominion Experimental Farms throughout Canada. These trials have been conducted annually for several years with the object of making careful and thorough comparisons of the agronomic characteristics of barley varieties. They constitute an excellent source of materials for malting studies. The seed used is authentic pedigreed stock which is distributed from the Central Experimental Farms, Ottawa. The barleys are grown in plots of five rod-rows arranged in a modified balanced block with quadruplicate plots for each variety. Marginal effects are reduced to a minimum by harvesting only the centre three rows. The samples for malting were obtained by bulking the grain harvested from quadruplicate plots.

* Two-rowed varieties, all others are six-rowed.

METHODS

The varieties were malted in two separate series, one for the smooth-awned varieties and the standard variety O.A.C. 21, and one for the rough-awned varieties. The barleys were split into two samples by means of a Boerner sampler, and the duplicate samples were then arranged in random order within each series.

Each sample was subjected to an assortment test (1) and the material remaining on the $5\frac{1}{2}/64$ in. and coarser sieves was bulked, weighed and reported as percentage plump barley. A sample representing 250 gm. of barley dry matter was used for malting. It was generally possible to obtain this from the plump barley but in certain cases it was necessary to make the sample up to weight with thin barley. The samples were malted in batches of twelve in the equipment described by Harrison and Rowland (5). The method used was that described by these authors except that: (a) the barleys were steeped to a moisture content of 44%, the time required being determined by duplicate pilot steeping tests; and (b) the last stage of the kilning was carried out at 80° C. rather than at 90° C. The malt yields were calculated from the weights of the sample before and after malting and the respective moisture contents of the barley and malt.

The twenty-four malts made each week were divided into duplicate samples with a Boerner sampler and then were analyzed in random order. Moisture, extract (fine grind) and colour were determined according to the Official Methods of the American Society of Brewing Chemists (1). Diastatic power was determined by a modification of the Official Method involving the use of a ferricyanide reagent (2). Notes were also taken on time of conversion, speed of filtration and on the clarity and odour of the wort.

RESULTS AND DISCUSSION

Varietal Differences

No differences between varieties were demonstrated with respect to colour, moisture, time of conversion, speed of filtration, clarity of wort or odour of wort, and for this reason the results of these determinations and observations are not reported.

The data on extract yield, diastatic power, malt yield and percentage plump barley are summarized in Table I. Each figure represents the difference between the mean values, over a group of either western or eastern stations, for the standard variety O.A.C. 21 and the variety listed in the first column. A positive sign indicates that the variety is superior to O.A.C. 21 and a negative sign that it is inferior. The necessary differences between means required for a 5% level of significance were calculated by statistical methods and have been included in the table.

The varieties did not fall in exactly the same relative position for each station, a fact which is amply illustrated by the differences between the values for western and eastern stations. As a result the differences between varieties which can be considered statistically significant are relatively large, but when such differences occur they show that the variety is fairly consistently inferior or superior to the standard variety at all stations. With respect to diastatic power and malt yield it must be admitted that the lack of precision of the test increased the necessary differences considerably.

TABLE 1.—DIFFERENCES BETWEEN MEAN VALUES FOR O.A.C. 21 AND OTHER VARIETIES

Variety	Extract, %		Diastatic power, °L.		Malting yield, %		Plump barley, %	
	West	East	West	East	West	East	West	East
Smooth-awned varieties								
Brandon 1099	−2.7*	—	−26*	—	+2.0	—	−2.3	—
Byng	—	0	—	−38*	—	+2.1*	—	+3.1
Newal	−1.9*	—	+40*	—	+1.2	—	−8.0	—
Nobarb	−1.8*	−0.8*	−15	−25*	+1.1	−0.8	−5.2	+0.8
Regal	−1.4*	−1.2*	−34*	−29*	+1.4	−0.7	−4.7	+5.8
Velvet	−1.5*	0	+ 2	−14*	+0.8	+0.1	+1.1	+6.8*
Wisconsin 38	−2.7*	−2.2*	−28*	−25*	+2.1	0	+9.2*	+10.1*
York	—	−0.3	—	−19*	—	+0.8	—	+0.8
O.A.C. 21	73.2	74.8	118	93	87.3	90.1	86.5	87.2
Necessary difference	0.7	0.7	25	8	2.3	1.8	8.8	6.6
Rough-awned varieties								
Olli	+1.5	+2.9*	+42*	+18	−3.8*	−2.3*	−10.9	−6.0
Peatland	+0.5	+1.0	+ 9	+10	−0.5	−2.0*	−3.5	−5.2
Pontiac	—	−0.2	—	− 4	—	0	—	−1.9
Victory†	+0.2	—	−19	—	−0.5	—	−8.6	—
Washington†	+0.1	+3.5*	+ 3	−14	0	+0.8	+16.4*	+3.7
O.A.C. 21	73.1	75.4	125	88	88.3	90.6	80.6	94.5
Necessary difference	1.9	1.5	35	28	2.4	1.9	11.7	9.4

* Differences which are statistically significant are marked with an asterisk.
† Two-rowed varieties, all others are six-rowed.

The most important of the malting qualities examined are extract and diastatic power, and it is assumed that in order to be considered satisfactory a variety should be equal to O.A.C. 21 with respect to these qualities. It is interesting to note that there is a clear-cut distinction between the smooth-awned and rough-awned varieties. All of the former are inferior to the standard variety with respect to one or both of the two important qualities, whereas the latter are all equal or slightly superior to the standard. Velvet, Byng and York, at eastern stations, compare favourably with the standard with respect to extract. The two most popular smooth-awned varieties, Wisconsin 38 and Regal, appear to have the poorest malting qualities. None of the smooth-awned varieties can be considered equal in malting quality to O.A.C. 21, but Newal might find a limited use for the production of malts of high diastatic power and may also be useful as a parent for breeding work.

Amongst the rough-awned group, Olli proved to be superior to O.A.C.21 in production of both extract and diastatic power. Peatland, Pontiac and the two-rowed variety Victory, can be classed as about equal to the standard. Washington, another two-rowed variety exhibits the true characteristic of this class by producing a high extract yield. It is doubtful, however, whether it is advisable to make direct comparisons between two-rowed and six-rowed varieties.

Statistical analyses of the data, reported in a later section, provide very strong evidence that varietal differences exist with respect to extract and diastatic power. Extensive studies on varietal differences in extract yield have been made by Bishop (3, and earlier papers) and these differences have been expressed quantitatively in terms of varietal constants for use with prediction equations. The existence of varietal differences in diastatic activity has been demonstrated by Thunaeus and Schröderheim (7) and by Bishop (4). Additional data bearing on this point have recently been published by Shellenberger and Bailey (6).

Although only four out of the twenty differences reported under malt yield proved to be significant, the data have been reported since they illustrate one of the limitations of the malting test and the difficulty of interpreting the data accurately. It will be observed that significant differences with respect to malt yield are associated with differences in diastatic power of opposite sign. The outstanding examples are those of Byng and Newal. Within certain limits a large malt yield is partly the result of less growth during the germination period. At first sight, it appears that the low diastatic power produced by Byng is the result of the poor response of this variety to the standard conditions under which the malt was grown. Under more favourable conditions, the variety would doubtless produce a higher diastatic power. There is, however, a different viewpoint. It seems probable that the poor growth is the result rather than the cause of enzyme deficiencies, and if this is the correct interpretation then inability to produce a reasonably high diastatic power may be considered a deficiency in malting quality which may express itself in poor growth and modification.

Only four of the differences listed under percentage plump barley proved to be significant. The necessary differences are large not because of lack of precision in the test but because the performance of the varieties was relatively unequal at different stations. This interaction between varieties and stations was probably increased by variations in cleaning and grading of the samples at the different stations before they were submitted to the malting laboratory. The data indicate that, in general, the smooth awned varieties were superior and the rough-awned varieties inferior to O.A.C. 21 with respect to percentage plump barley suitable for malting.

Differences Between Stations

Five varieties, Nobarb, Regal, Velvet, Wisconsin 38 and O.A.C. 21 were grown at all thirteen stations. The mean values for each station, over the five varieties, for extract and diastatic power of the malt, and for the protein content of the barley, are reported in Table 2. The stations have been listed in ascending order with respect to extract.

Although the data are of some interest because they give an indication of the wide variety of environmental conditions under which the varieties were tested and of the effect of this on the quality of the malt, they are reported mainly because they illustrate the relations which exist between extract, diastatic power and protein. The correlation and regression coefficients for the three pairs of determinations are reported in Table 3. It will be observed that all three correlations are highly significant.

The inverse correlation between protein content of barley and the extract yield of the malt made from it has been known to exist for some

TABLE 2.—MEAN VALUES FOR EACH STATION OVER FIVE VARIETIES

Stations	Malt		Barley protein,* %
	Extract %	Diastatic power, °L.	
Scott	69.1	146	—
Manitoba Agricultural College	70.6	88	12.0
Ontario Agricultural College	71.0	82	—
Brandon	71.8	108	12.8
Lennoxville	72.5	84	12.6
Ottawa	72.6	92	13.2
Indian Head	72.8	91	11.8
University of Alberta	73.6	92	12.4
Ste. Anne de la Pocatiere	74.4	80	10.6
Macdonald College	74.6	82	10.8
Nappan	75.1	62	9.8
Fredericton	75.2	65	9.7
Charlottetown	76.2	47	8.6

* Nx 6.25; 13.5% moisture basis.

TALBE 3.—COEFFICIENTS OF CORRELATION AND REGRESSION

Correlation between	Correlation coefficient		Regression coefficient
	Found	Required for P = 0.01	
Extract — protein	−0.852	0.735	— 0.96
Diastatic power — protein	.916	.735	10.4
Diastatic power — extract	— .827	.684	— 9.6

time and has been thoroughly investigated by Bishop (3, and earlier papers). That protein and diastatic power are directly correlated has also been widely recognized by practical maltsters and Bishop (4) has recently used prediction equations for diastatic power based largely on this correlation. The development of such equations for Canadian barley varieties will be attempted when a more comprehensive and precise set of data have been accumulated.

Statistical Analyses

Separate series of analyses of variance were made for: (a) the seven smooth-awned varieties grown at five western stations and Ottawa; (b) the seven smooth-awned varieties grown at eight eastern stations; and (c) the four rough-awned varieties grown at eleven stations. In each series separate analyses were made for extract, diastatic power, malt yield and percentage plump barley. The variance in each case was analyzed into portions due to: (i) average differences, over all stations, between varieties; (ii) average differences, over all varieties, between stations; (iii) differences in the relative performance of the varieties at different stations; and (iv) differences between duplicate maltings. The results of the analyses are reported in Table 4. The necessary differences required for a 5% level of significance, reported in Table 1, were calculated from the mean squares due the interaction between varieties and stations.

TABLE 4.—ANALYSES OF VARIANCE

Variation due to	Degrees of freedom	Mean squares			
		Extract, %	Diastatic power, °L.	Malt yield, %	Plump barley %
Smooth-awned Varieties, Western Stations					
Varieties	6	10.034**	4,184**	6.20	374.60*††
Stations	5	40.936**	4,913**	12.31††	722.27**
Varieties × stations	30	.729††	926††	8.06††	117.79††
Duplicates	42	.282	284	3.55	.97
Smooth-awned Varieties, Eastern Stations					
Varieties	6	11.122**	2,172**	24.69**	195.22*††
Stations	7	45.298**	2,499**	16.12*††	2,750.33**
Varieties × stations	42	1.042††	110	6.45	80.69††
Duplicates	56	.244	110	5.16	1.31
Rough-awned Varieties, All Stations					
Varieties	3	25.241**	5,638**	33.93**	715.01*††
Stations	8	51.804**	13,463**	32.84**	641.46**
Varieties × stations	24	3.506††	1,202††	5.60	129.51††
Duplicates	36	.166	206	5.26	2.61

Double signs denote that the mean square attains a 1% level of significance, single signs denote a 5% level.
* and **, Significantly greater than the mean square due to varieties × stations.
† and ††, Significantly greater than the mean square due to duplicates.

The data substantiate statements made previously in this paper with respect to varietal and station differences. With one exception, the mean squares due to varieties and to stations are significantly greater than the corresponding mean squares due to the interaction.

The mean squares due to the interaction between varieties and stations, for extract, diastatic power and malt yield, are all significantly greater than the corresponding mean squares due to differences between duplicate maltings. In other words, the varieties were not placed in exactly the same relative position at different stations. This was hardly to be expected. It seems apparent, for example, that early and late maturing varieties such as Olli and O.A.C. 21 cannot be expected to maintain the same relative positions irrespective of whether the environmental conditions do or do not favour an early variety. The interaction effect undoubtedly constitutes the greatest difficulty in comparing varieties. It seems probable, however, that when the differences between two varieties are so great as to be of practical importance, they will not be masked by the interaction effect.

SUMMARY

Malting tests were made on samples of fourteen varieties of barley, each of which was grown in 1935 at from four to thirteen experimental stations in Canada. All samples were malted under the same conditions.

Not one of the eight smooth-awned six-rowed varieties, Brandon 1099, Byng, Newal, Nobarb, Regal, Velvet, Wisconsin 38 and York, produce both as high an extract yield and diastatic power as the standard malting

variety O.A.C. 21. Byng, Velvet and York, at eastern stations only, compared favourably with the standard in extract yield, while Newal was characterized by very high diastatic power. The rough-awned six-rowed varieties, Peatland and Pontiac, and the two-rowed varieties, Victory and Washington, proved to be about equal to the standard in production of extract and diastatic power, while Olli proved to be superior in both respects.

Study of the mean values over five varieties, for each of 11 stations, show that protein content of barley and extract yield of malt are inversely correlated, and that protein content and diastatic power are directly correlated. The correlation coefficients were found to be 0.852 and 0.916, the required value for P = 0.01 being 0.735.

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Résumé

Études sur la qualité de maltage. 1. Essais de variétés de 1935—J. Ansel Anderson et Harry Rowland, Laboratoire national de recherches et Université du Manitoba.

Des échantillons de quatorze variétés d'orge, qui toutes avaient été cultivées en 1935 sur un nombre de stations expérimentales variant de quatre à treize, ont été soumis à des essais de maltage. Les conditions de maltage étaient les mêmes pour tous les échantillons. Aucune des huit variétés à six rangs et à barbe lisse, Brandon 1099, Byng, Newal, Nobarb, Regal, Velvet, Wisconsin 38 et York, n'a produit un rendement d'extrait et une puissance diastasique aussi élevés que la variété régulière O. A. C. No. 21. Aux stations de l'Est seulement, les Byng, Velvet et York, ont soutenu avantageusement la comparaison avec le type-modèle au point de vue du rendement d'extrait, tandis que la Newal s'est caractérisée par une puissance diastasique très élevée. Les variétés à six rangs et à barbe grossière, Peatland et Pontiac, et les variétés à deux rangs, Victoire et Washington, se sont montrées à peu près égales au type-modèle dans la production d'extrait et de puissance diastasique, tandis que la Olli s'est montrée supérieure sous les deux rapports. L'étude des valeurs moyennes sur cinq variétés, sur chacune des 11 stations, indique que la teneur en protéine de l'orge et le rendement d'extrait de malt sont reliés de façon inverse, tandis que la quantité de protéine et la puissance diastasique sont directement reliées Le coefficient de corrélation était de 0.852 et 0.916 et la valeur requise pour P = 0.01 est de 0.735.

STUDIES ON RHIZOCTONIA SOLANI KÜHN.

II. EFFECT ON YIELD AND DISEASE OF PLANTING POTATO SETS INFESTED WITH SCLEROTIA[1]

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In the previous paper (5) the effective transfer, during the first six weeks, of *Rhizoctonia Solani* from sets heavily infested with viable sclerotia, was discussed in the light of the infection from apparently clean untreated sets, and of that arising from the natural soil infestation. It was proved that, in general, both severity and frequency of stem lesions were increased as a result of untreated sclerotia on the sets. But extreme variability among the 34 experiments persisted, both in severity of lesions and in percentage of plants which escaped. An average of only 42% of all plants which grew from infested sets had stem lesions. Of this, approximately 29% were traceable to the sclerotia, and 13% to the natural soil infestation.

This report attempts to deal with the effect of sclerotia infested sets on yield, and on the occurrence of sclerotia on the new crop at harvest, and to evaluate, in so far as the experimental data will permit, the relative merits of using large-size tubers, small-size tubers, or total yield as criteria for indicating the effects of treatment. An attempt was also made to determine whether there is a marked tendency for stem lesions and reduced yield to be correlated.

MATERIALS AND METHODS

Arrangement and Location of Experiments

The variety used in the 1933, 1934, 1935 and 1936 tests was certified Early Ohio. Each year the material was from a different source. During the first three years each test consisted of two series, designated as "A" and "X". The sets for the A series were from apparently clean tubers treated for five minutes in the standard acid solution (1–500) of mercuric chloride, and quickly dried. The sets for the X series carried a heavy infestation of sclerotia and were untreated. There were, in all, during the first three years, 27 separate but uniform tests with the two series indicated. Each series consisted of 100 sets. The sets of each series were alternately planted 20 inches apart. In 1936 another series, namely "O", in which the sets were apparently clean and untreated, was added in order to obtain a better check on the possible depressing effect of treatment on the yield. Nine separate but uniform tests were completed in that year, all being in the immediate vicinity of Edmonton. The 27 tests of the three previous years were located at several of the Dominion experimental stations across Canada, on the experimental plots at the University of Alberta, Edmonton, and on several private farms near Edmonton. The location, soil type, and crops preceding each of the 27 experiments indicated, except 1936,

[1] Contribution No. 505 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada, co-operating with the Univeristy of Alberta.
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may be obtained by referring to the corresponding numbers in Table 1 of the previous study (5).

TABLE 1.—YIELD OF POTATOES AS AFFECTED BY PLANTING SETS (X SERIES) HEAVILY INFESTED WITH VIABLE SCLEROTIA: 1933, 1934 AND 1935 EXPERIMENTS

Exp.	Yield per series**—kg.								Ratio large to total %		Per cent severity lesions stems 6 wks.	
	Large size			Total			Small size					
	A	X	t value*	A	X	t value*	A	X	A	X	A	†
1933												
1	66.5	70.5	.8	78.6	91.5	2.5	12.1	21.0	85	77	28.9	1
2	63.9	60.1	2.8	75.5	74.7	.2	11.6	14.6	85	80	17.7	1‡
5	70.8	63.5	1.6	76.9	77.4	.1	6.1	13.9	92	82	2.5	4
6	18.2	17.3	.5	24.4	21.6	1.8	6.2	4.3	75	80	.7	16
7	22.1	18.6	1.3	37.3	39.9	1.2	15.2	21.3	59	47	9.4	2
8	30.8	36.9	.2	35.3	42.3	2.7	4.5	5.4	87	87	3.5	6
10	51.1	42.2	2.7	71.6	68.4	.7	20.5	26.2	71	62	.8	10
11	7.4	9.3	1.1	20.7	22.9	1.3	13.3	13.6	36	41	2.9	9
1934												
12	64.1	56.7	1.8	96.8	95.6	.3	32.7	38.9	66	59	6.9	1‡
13	13.7	17.0	2.0	29.7	33.0	2.0	16.0	16.0	46	51	.4	3
14	73.5	55.8	3.3	89.3	112.4	.4	15.8	56.6	82	50	.3	4
15	64.0	50.2	3.3	73.4	66.8	1.5	9.4	16.6	87	75	4.1	9
16	67.1	57.1	2.2	80.2	76.0	.9	13.1	18.9	84	75	3.3	14
17	55.2	47.2	2.0	64.8	65.8	.2	9.6	18.6	85	72	11.0	20
18	16.4	19.7	1.8	30.9	35.5	2.7	14.5	15.8	53	55	17.7	2
19	11.7	14.9	2.1	19.8	24.5	3.4	8.1	9.6	59	61	3.5	15
20	69.0	64.3	1.1	91.6	86.5	1.2	22.6	22.2	75	74	2.4	1‡
21	65.2	50.7	4.6	82.0	66.3	5.0	16.8	15.6	79	76	3.2	33
22	29.6	36.5	1.6	36.9	49.2	.2	7.3	12.7	80	74	.2	3
1935												
23	73.3	71.1	.4	78.9	95.9	3.4	5.6	24.8	93	74	1.4	30
27	67.4	66.1	.2	77.4	91.2	2.9	10.0	25.1	87	72	10.9	4
28	14.3	20.9	3.1	24.3	33.7	5.5	10.0	12.8	59	62	3.8	5
29	25.9	23.5	1.3	30.3	31.1	.5	4.4	7.6	85	75	5.8	17
30	19.1	16.7	1.2	45.9	45.0	.4	26.8	28.3	42	37	1.7	7
32	14.4	23.1	5.9	24.6	37.6	8.4	10.2	14.5	58	61	.1	6
33	35.4	29.3	2.8	48.8	46.7	1.0	13.4	17.4	72	63	.8	23
34	42.8	40.6	.7	55.0	53.8	.4	12.2	13.2	78	75	.2	30
Av.	42.7	40.0		55.6	59.0		12.9	18.7				

* 0.6 or greater indicates significance.
** A, treated clean sets; X, sclerotia infested sets.
† Increase in severity of infection of stems in the X series over that in the A series at the six-weeks stage. See (5) Table 1, Studies in *Rhizoctonia Solani*. I.
‡ Decrease.

The Taking of Data

For the 1933, 1934 and 1935 tests the weight in grams of yield and large-size tubers was recorded for each hill. In 1936 it was recorded in ounces. Units bordering on missing hills were discarded. The presence of sclerotia was noted. A record of plants with stems lesioned, deformed tubers, and rhizoctonia symptoms was made for the 1936 tests only. Data were not taken on lesions of the stolons.

Analysis of the Data

The yield data of the tests prior to 1936 were statistically analyzed by a formula recommended by Dr. C. H. Goulden, senior cerealist, Dominion Rust Research Laboratory, Winnipeg. The t value obtained from Fisher's tables (2) is employed to indicate significance of difference. Owing to a different plot technique the yield data of the 1936 tests were treated by Fisher's analysis of variance method, and the significant difference ($2 \times \sqrt{2} \times SE$) for each test expressed in ounces.

EXPERIMENTAL RESULTS

The 1933 - 1935 Data

According to the hypothesis that the rhizoctonia disease decreases total yield, and increases the proportion of small tubers, the yield of large-size tubers in the A series should have been fairly consistently higher than in the corresponding X series. Also, the yield of small-size tubers should have been consistently higher in the X series than in the A series. Finally, this should have been especially noticeable in those experiments in which there was a marked increase of the disease on the stems of the X series as determined at the six-weeks stage of growth. Let us examine the data in Table 1.

Large-size Tubers

Significant increases in yield of large-size tubers occurred in the A series (clean treated sets) in fifteen of the twenty-seven tests listed, namely, Nos. 2, 5, 7, 10, 12, 14, 15, 16, 17, 20, 21, 29, 30, 33 and 34. Significant reductions are recorded for eight cases, namely, Nos. 1, 11, 13, 18, 19, 22, 28 and 32. It was not significantly different from the yield of the X series in four tests (Nos. 6, 8, 23 and 27, columns two and three).

Total Yield

Significant differences in total yield were obtained in all but nine (Nos. 2, 5, 12, 14, 17, 22, 29, 30 and 34) of the 27 experiments. In seven tests (Nos. 6, 10, 15, 16, 20, 21 and 33) they were significantly greater in the A series, and in the remaining eleven (Nos. 1, 7, 8, 11, 13, 18, 19, 23, 27, 28 and 32) significantly greater in the X series (Table 1).

Yield of Small-size Tubers

Regarding yield of small-size tubers in the A and X series (statistically significant values not determined), it is sufficiently obvious from a comparison of columns eight and nine of Table 1, that the yield of this size in the X series was noticeably greater in approximately 14 of the 27 tests (Nos. 1, 5, 7, 10, 12, 14, 15, 16, 17, 22, 23, 27 and 32), or about one-half of them. In the remaining 13 tests the listed difference, although in all but two instances slightly greater in the X series, was not significant. The ratio of yield of large-size tubers to total yield, expressed in percentage of total yield (columns ten and eleven), provides the same general picture just described.

Relation of Stem Lesions at the Six-weeks Stage to Yield

There remains to examine whether a high disease rating on the stems of the X series at the six-weeks stage is proportionately reflected in reduced yield of large-size tubers, and an increased yield of small-size tubers in this series. Best examples for the purpose would be those cases where

the rating was high (9 to 36%) on the X series, and low (0.2 to 3.5%) on the corresponding A series. Thus, in the nine experiments (Nos. 6, 10, 11, 16, 19, 21, 23, 33 and 34), the yields of large-size tubers were greater in the A series in five cases, and less in the other four. In Nos. 1, 2, 7, 12, 17, 18, 27 and 29, where the disease rating was similar and fairly high in both series, the yields in the A series were greater in five out of eight instances. Finally, in the remaining ten tests (Nos. 5, 8, 13, 14, 15, 20, 22, 28, 30, and 32), where the ratings for the A series varied between 0.1 and 4%, and between 4 and 9% for the X series, the yields in five instances were significantly greater in the A series, and either less, or not greater, in the other five. Obviously the rating on the stems at the six-weeks stage was not consistently reflected in the yields of large-size tubers at harvest.

The relation to small tubers was not constant, for in five out of nine cases (Nos. 6, 10, 16, 21 and 33) the yield of the small-size was significantly less in the X series, while in the remaining four tests indicated, it was significantly greater. Similarly inconstant behaviour is indicated by a number of the remaining experiments.

Sclerotia

Data on sclerotia on the tubers at harvest are not included because throughout the 27 experiments their occurrence was practically negligible.

Summary of 1933–1935 Experiments

The main points from the 1933–1935 data may be briefly stated. Neither large-size nor small-size tubers, or total yield, were dependable criteria to detect the effect from planting sclerotia-bearing sets. The ranking reliability of each criterion, as deduced from these tests, would appear to be in the order mentioned. The first two gave expected results in approximately 50% of the tests, and the third in 25% of them. Also, results indicated that degree of severity of stem lesions at the six-weeks stage was not closely correlated with yield values at harvest.

THE 1936 DATA

A third series, namely "O", the sets of which were clean and untreated, was added to series A and X in 1936, to obtain a better check on the possible depressing effect of the treatment on the yield. Additional data were also secured, such as yield of plants with stems lesioned, and also yield of plants with stems unlesioned at harvest, and the association of deformed tubers, or sclerotia, with plants having lesioned or unlesioned stems. The data from the nine separate, but uniform, tests, are in Tables 2, 3 and 4.

Relation of Sclerotia on Sets to Yield

The yields of both large-size, small-size tubers, and also the total yields were remarkably similar in the A, O and X series in each of the nine experiments. Concerning the large-size tubers, the yields in the A series (clean treated sets) were uniformly less than in the X series (sclerotia on sets). However, in all but two tests (Nos. 4 and 9, Table 2) the differences were not statistically different. The yields obtained from the O series in four tests were slightly less than from the corresponding A series, and slightly greater in the remaining five tests, but none of them were statistically significant. The average of the A series for the nine tests

TABLE 2.—EFFECT OF PLANTING SETS HEAVILY INFESTED WITH VIABLE SCLEROTIA OF *Rhizoctonia Solani* ON: LARGE-SIZE TUBERS; SMALL SIZE-TUBERS; AND TOTAL YIELD, IN 1936

Exp.	Average yield, ounces, per series* of 10 replicates											
	Large				Small				Total			
	A	O	X	†	A	O	X	†	A	O	X	†
1	330.4	366.5	350.9	30.4	9.3	8.3	14.6	6.8	339.7	374.8	365.5	29.2
2	215.6	208.6	219.5	15.7	4.8	8.2	9.2	3.8	220.4	216.8	228.7	16.1
3	423.1	423.3	442.1	39.7	14.9	13.9	22.0	10.8	438.0	437.2	464.1	39.3
4	367.9	356.2	415.0	31.3	8.6	7.5	12.0	5.8	376.5	363.7	427.0	31.5
5	313.3	312.0	331.2	33.9	11.9	16.1	16.6	6.0	325.2	328.1	347.8	35.0
6	123.3	141.3	136.9	24.9	18.9	17.2	21.2	1.7	142.2	158.5	158.1	34.2
7	339.6	353.7	357.2	26.9	7.5	9.2	10.1	4.7	347.1	362.9	367.3	28.0
8	309.3	319.4	318.1	31.3	5.5	9.1	12.0	4.3	314.8	328.5	330.1	28.2
9	242.8	279.5	288.5	42.9	3.7	6.6	5.5	6.1	249.0	290.5	297.6	77.0
Average	296.1	306.7	317.7		9.4	10.7	13.7		305.9	317.9	331.8	

* A, clean sets treated; O, clean sets untreated; X, sclerotia infested sets.

† $2 \times \sqrt{2} \times$ SE (statistically significant difference).

again indicated a trend toward less yield of large-size tubers. For total yield, the situation was not materially different from that indicated for yield of large-size tubers. The trend was again toward greater yields in the X series than in the A series, but with a statistically significant difference only in the case of test No. 4.

The yield of small tubers again appears to have shown a tendency to increase in the X series over that in the A series. Significant differences were recorded in experiments Nos. 2 and 8, and near significance in experiment No. 5. Also, the recorded yields in the O series were less than in the X series in all tests but one, but they were not significantly different. With reference to treatment, there might have been a tendency for the treatment to decrease yields in the A series in a few instances, but nothing of a definite nature is evident from the data.

Percentage Stems Lesioned

In columns 2, 3 and 4 of Table 3 are listed the percentages of stems lesioned under each of the three series, and the decrease or increase for the X series over the A series indicated for each experiment. In three cases (Nos. 4, 7 and 8) there were decreases of 20, 10 and 8%, respectively, but in the other six, increases ranging from 14 to 27% were recorded. A satisfactory explanation for the relatively large decrease mentioned for tests Nos. 4, 7 and 8 appears to be lacking. Possibly it was caused by irregular soil infestation.

An average of only 43% of the plants in the X series of the nine tests had stem lesions at harvest, despite the fact they had grown from sclerotia-infested sets. It will be recalled (5) that in the same series of the 34 tests made during 1933, 1934 and 1935, an average of 42% of the plants had stem lesions at the six-weeks stage. Thirty-four hundred plants were involved in the latter instance, and 900 in the former.

Yield from Lesioned versus Unlesioned Stems

The average yields of all plants in each series having lesioned stems, and of those with unlesioned stems, are recorded in columns 6 to 13 of Table 3. Under both headings there is a trend, fairly consistent and general, yet probably not significant, for the yield in the X series to be slightly greater than in the A series. This trend is more evident for the case of the lesioned stems. However, the averages for each experiment (columns 9 and 13) are remarkably similar in all tests except Nos. 4, 8 and 9. Therefore, the available evidence suggests that the yield from lesioned plants was at least approximately equal to that of the plants with unlesioned stems under the conditions of the experiment.

TABLE 3.—EFFECT OF PLANTING SETS HEAVILY INFESTED WITH SCLEROTIA OF *Rhizoctonia Solani*, ON PERCENTAGE OF PLANTS WITH STEM LESIONS; AND ON AVERAGE TOTAL YIELD PER PLANT FROM STEMS LESIONED, AND FROM STEMS UNLESIONED, 1936

Exp.	Per cent plants stems lesioned per series*				Average total yield per plant, ounces							
					Stems lesioned				Stems unlesioned			
	A	O	X	†	A	O	X	Av.	A	O	X	Av.
1	32	36	48	+16	34.3	36.3	37.3	36.1	33.8	37.8	36.0	35.8
2	25	42	52	+27	23.7	21.6	22.5	22.4	21.5	21.8	22.8	21.9
3	42	43	60	+18	43.6	43.7	49.6	46.0	34.6	43.8	42.1	43.3
4	68	64	48	−20	36.1	36.7	40.1	37.4	41.3	35.8	45.1	41.3
5	29	47	43	+14	32.0	33.1	35.4	33.7	32.7	32.5	34.3	33.1
6	28	47	53	+25	15.9	13.9	15.0	14.8	13.6	17.6	16.7	15.5
7	38	27	28	−10	32.6	34.9	40.0	35.5	36.0	36.8	35.5	36.1
8	32	38	24	− 8	29.1	29.9	30.1	29.7	32.5	34.0	33.8	33.7
9	17	27	31	+14	19.8	25.7	27.3	25.0	27.0	31.9	29.0	29.2
Average	34	41	43		29.7	30.6	33.0	31.1	31.3	32.5	32.8	32.2

* A, clean sets treated; O, clean sets untreated; X, sclerotia infested sets.
† Difference between X and A series.

Deformed Tubers

Concerning the effect of planting sclerotia-bearing sets on deformed tubers, the data in column 2, 3 and 4 of Table 4 indicate no real difference between the number of plants in this category in experiments Nos. 1, 2, 4, 5, 6 and 7. Regarding Experiments Nos. 3, 8 and 9, the data in columns 5 and 6 indicate the differences listed are certainly not significant. For example, in test 8 there were 37 hills of deformed tubers under plants with unlesioned stems, and only 15 under the lesioned series. The corresponding figures for experiment No. 9, are 12 and 7, and for experiment No. 3, they are 25 and 29. The totals for all nine experiments are 130 hills of deformed tubers from lesioned plants, and 174 hills from those lesion-free. Thus, under the conditions of these experiments, there was no evidence that hills of deformed tubers were necessarily associated with lesioned stems. This is further confirmed by comparing the corresponding data in columns 3, 4 and 5 of Tables 3 and 4.

Sclerotia

The results on occurrence of sclerotia on the tubers of plants in A, O and X series, and possible association with lesioned or unlesioned plants,

TABLE 4.—EFFECT OF PLANTING SETS HEAVILY INFESTED WITH VIABLE SCLEROTIA OF *Rhizoctonia Solani* ON: PLANTS HAVING DEFORMED TUBERS; PLANTS WITH SCLEROTIA ON TUBERS; AND MISSING PLANTS, 1936

Exp.	Plants with deformed tubers					Plants with sclerotia on tubers					Plants missed from series		
	From series			From stems		From series			From stems				
	A	O	X	Lesioned	Unlesioned	A	O	X	Lesioned	Unlesioned	A	O	X
1	2	3	3	5	3	2	2	3	3	4	1	1	1
2	5	9	5	9	10	—	—	—	0	0	—	—	—
3	11	19	24	29	25	1	1	—	0	2	—	1	—
4	9	4	12	15	10	—	—	—	0	0	1	—	1
5	4	3	3	4	6	1	3	1	2	3	—	—	2
6	25	25	26	25	41	—	1	7	6	2	—	—	2
7	24	12	15	21	30	16	1	—	9	8	—	—	1
8	13	18	21	15	37	6	3	3	6	6	1	1	1
9	4	5	10	7	12	2	1	3	2	5	—	—	—
Total	97	98	119	130	174	28	11	17	28	30	3	3	8

are given in columns 7 to 11 in Table 4. In all but experiment No. 7 the hills of sclerotia-bearing tubers were about equally distributed in the three series. It is interesting to note that in the nine experiments, from 900 plants in each of the A, O and X series, or a total of 2700 plants, only 58 plants had sclerotia on the tubers. Of these, 28 had lesioned stems, and 30 were lesion-free. The obvious conclusion here is that the conditions throughout were unfavourable to formation of sclerotia. The general presence of the pathogen in the soil was demonstrated by the fairly high percentage of lesions on the stems of all series. The large amount of sclerotia on the X sets did not increase sclerotia on the new crop.

Missing Plants

Regarding the effect of planting sclerotia-bearing sets on missing plants, the data in the last three columns of Table 4 indicate that out of a total of 2700 plants only 14 failed. Of these, eight were in the X series, and three in O and A, respectively. Obviously the data are too meagre for conclusions.

Effect of Treatment on Yield

During the first six weeks of growth a noticeable decrease in vigour of plants in the treated A series was observed in 1933 and 1934. It was not evident in the 1935 and 1936 experiments.

The trend toward reduced yield in the A series, which was most noticeable in the 1936 experiments, and less so in those of the previous years, has been discussed. Theoretically, the trend should have been in favour of the A series. It will also be observed in Table 2, that in 1936 the yields from the O series (clean untreated sets) were, in general, very similar to those from the A series. The spread was between the A and X series.

Therefore, the effect of the two standard mercuric chloride tuber treatments on yield was studied in 1936. One lot of clean tubers was soaked for one and one-half hours in strength 1–1000 $HgCl_2$ solution, and another lot for five minutes in strength 1-1000, to which was added one per cent by volume hydrochloric acid. The control was not treated. Each

of the three series consisted of 27 randomized replicates, each of 10 hills, or 270 hills for each test.

The yields from the control series (Table 5) were greater than those from either of the other two series. The difference approached statistical significance in the case of the acid series (1–1000 $HgCl_2$), and the control, but not so for the non-acid (1-1000 $HgCl_2$) series. In other words, both treatments tended to reduce the yields, the acid dip being slightly the more effective. Hence, the results of this test suggest that the acid treatment (1–500 $HgCl_2$), used for the A series of the main 1936 experiment, might have slightly depressed the yield of this series. However, all of the spread between A and X series cannot be accounted for by treatment, since the trend continued from the untreated O series toward the untreated, sclerotia-bearing X series.

DISCUSSION

It is well to bear in mind that the foregoing results were obtained from 36 experiments during four years, under diverse natural field conditions, which included a very wide range of soil type, culture, and previous crop history (5). In so far as material is concerned, the results are based on one difference, namely, one series of treated clean tubers versus another series of untreated tubers, heavily infested with sclerotia. The question of the effectiveness of several chemicals is excluded.

TABLE 5.—EFFECT ON YIELD OF TREATING CLEAN POTATO TUBERS WITH ACID MERCURIC CHLORIDE SOLUTION AND WITH UNACIDIFIED SOLUTION, 1936

Treatment solution	Average yield, ounces, of 27 replicates*				
	Total	$2 \times \sqrt{2} \times SE$	Large	$2 \times \sqrt{2} \times SE$	Small
$HgCl_2$, 1–1000, $1\frac{1}{2}$ hrs.	310.5		302.4		8.1
$HgCl_2$, 1–1000 + 1% HCl, 5 min.	303	24.5	294.7	24.6	8.3
Check	327		317.2		9.8

* Each 10 hills.

These experiments have shown that, in general, neither yield of large-size, nor small-size tubers, nor total yield, can be relied upon in average field tests to give expected results regarding the relative value of tuber treatments to control the rhizoctonia disease. The first two criteria gave results in about 40% of the experiments, and the latter in 20% of them.

It is perhaps natural that "total yield" proved the less reliable, since yield of small-size tubers is included. Small-size tubers may be the result of many other factors in addition to the rhizoctonia disease. Add to this the complicating factors of the natural soil infestation. Also, it is possible that, under certain conditions of soil moisture and available food, the pathogen, in pruning off certain stolons, may perform either a beneficial or a detrimental service; that is, increasing or decreasing the proportion of yield of large-size tubers to the small-size, or increasing the total yield. From other experiments not reported here, a very definite increase of both

large-size and total yield was obtained by amputating the first crop of stolons. This is mentioned to support the suggestion that the activity of the pathogen may have been partially responsible for the apparent trend toward higher yields in the sclerotia-bearing series of the nine experiments of 1936. Also, there is the complicating factor of the escape (judged on the basis of stem lesions) of a fairly high percentage of plants. Out of a total of 3,400 plants in the X series, approximately 58% of them had no lesions on the stems.

Finally, neither deformed tubers nor sclerotia on the tubers at harvest time were reliable indications of the benefit from tuber treatment. This is because the sclerotia were of extremely rare occurrence, and because the degree of severity of the latter indicated no constant correlation with reduced yield. A better understanding of the situation might have been possible if, during tuberization, the severity of the disease on the stolons had been determined.

In spite of the foregoing results, it is readily admitted that, under certain conditions, definite results would occur as expected with regard to sclerotia, yield of large-size, and small-size tubers, total yields, and possibly stem lesions. But, in view of the indefinite or contrary results in yield in approximately one-half of the 36 tests reported herein, the difficulty of always getting well defined results, and in the direction expected, with this pathogen, becomes obvious, in ordinary field experiments of this kind. If the sclerotia on the new crop are used as a criterion, the difficulty would appear to be very great under a wide range of conditions. Conditions favourable for definite experimental results obviously would include a soil practically free from natural infestation, temperature and moisture of the soil favourable to the growth of the pathogen from the sclerotia on the sets to the sprouts, and, later in the season, to the stolons. Finally, sclerotia from pathogenic strains of *R. Solani* must be on the sets. The temperature of the soil during the first month, at least, must have been fairly favourable for the disease throughout, since practically all the plantings were about May 12th. Soil thermograph readings at Edmonton indicated a range of from 15 to 23° C., during the period indicated.

A number of workers have discussed what data are most suitable to indicate, in field trials, the effect of tuber treatment. Dana (1) classified the yield at harvest according to marketable and total yield, and percentage of sclerotia-free tubers. He also observed that the (mercuric chloride solution) treatment appeared to depress the yield in certain experiments. Goss and Werner (3) used "total yield", and graded it according to percentage by weight of sclerotia-bearing and of clean stock. They pointed out the frequent scarcity of sclerotia in eastern Nebraska. They also reported the complicating effect on their experiments of the natural soil infestation, as indicated by stem infection of plants from "healthy seed". Raeder and Hungerford (4), in testing many chemical tuber treatments, state: "Sclerotia on the tuber surface at harvest time were the basis of disease readings, rather than stem lesions during the growing season". They, using Early Ohio, also stated: "In the Palouse district, in the vicinity of Moscow, Idaho, very few stem lesions ordinarily develop." Interesting problems which others have encountered could be cited, but space will not permit.

The results of the present studies apply particularly to field tests of certain chemical tuber treatments, some of which differ only slightly in effectiveness. The lethal effect of any of the treatments could be determined quickly, and at relatively small cost in the laboratory, where size (6) and texture of the sclerotia are considered. Its effect on the vitality of the tuber can be determined in the greenhouse, where disease and other complicating factors are practically absent. Also, it should be possible, and preferable, to determine there whatever protective value the treatment may possess from residual properties.

SUMMARY

The effect on yield and disease of planting potato sets infested with sclerotia of *Rhizoctonia Solani* was studied during four years in 36 experiments, under a wide range of soil types and crop sequences. In general, the use of yield of large-size tubers, or small-size tubers, or total yield, or yield of deformed tubers, or sclerotia on tubers at harvest proved to be undependable criteria to determine the relative value of tuber treatments. Although percentage of stem infection would be fairly reliable to indicate both soil infestation and control by a treatment during the early growth period, it is important, if not essential, to determine the effect of the disease on the stolons, if control of the disease is to be measured in terms of yield. The yield of large-size tubers was the most reliable of all criteria used. It gave expected results in about 40% of the experiments. An average of approximately 58% of the plants from the sclerotia-bearing sets had no disease lesions on the stems.

The laboratory method of determining the relative lethal effect on sclerotia and general suitability of various tuber treatments is suggested in preference to ordinary field tests.

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Résumé

Études de Rhizoctonia Solani Kuhn. 11 L'emploi de plantons de pommes de terre infestés de sclérotes, et l'effet de ces plantons sur le rendement et la proportion de maladie. G. B. Sanford, Dom. Laboratory of Plant Pathology, Edmonton, Alta.

L'effet exercé par les plantons infestés de sclérotes de *Rhizoctonia Solani*, au point de vue du rendement et du nombre de tubercules malades, a été étudié pendant quatre ans, dans trente-six expériences qui couvraient un grand nombre de sols de types différents et de successions de récoltes. En général, on a constaté que le rendement de gros tubercules, ou de petits tubercules, ou le rendement total, ou le rendement de tubercules difformes, ou de sclérotes sur les tubercules à l'arrachage est un critérium peu sûr pour déterminer la valeur relative des traitements. Le pourcentage d'infection de la tige indique assez exactement l'infection du sol et l'effet du traitement appliqué pendant la première période de végétation, mais il est important, sinon essentiel, de déterminer l'effet de la maladie sur les stolons si le contrôle de la maladie doit être étudié sur une base de rendement. Le rendement de gros tubercules a été le plus sûr de tous les critériums employés. Il a donné les résultats attendus dans 40 pour cent environ des expériences. Une moyenne d'environ 58 pour cent des plantes provenant de plantons qui portaient des sclérotes n'avaient pas de lésions de maladie sur les tiges. On recommande la méthode de laboratoire de préférence aux essais ordinaires dans le champ, pour déterminer l'effet léthal relatif des sclérotes et l'utilité générale des différents traitements appliqués aux tubercules.

A PROBABLE CULTURAL CONTROL FOR THE PALE WESTERN CUTWORM, *AGROTIS ORTHOGONIA* MORR.

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Since 1911 the pale western cutworm (*Agrotis orthogonia* Morr.) has been a serious pest of crops on the Canadian Prairies. Extensive experiments have been conducted in attempts to find a successful control for this insect. Since 1921 a prevention of infestation in summer-fallowed fields has been practised with exceptionally good results, and since 1923 outbreaks have been forecasted with reasonable accuracy. While these two measures have served to reduce greatly the annual damage caused by pale western cutworm, the losses to crops seeded on stubble or on land which has received drifted soil have continued unabated.

Field observations made during the last fifteen years have shown that occasionally there was some combination of circumstances which appeared to control the insect, and attempts have been made to determine these circumstances. A recent study of the insect in detail has shown that the eggs hatch as soon as the frost is out of the top two inches of soil, provided there is moisture present. The newly-hatched larvae may live for several weeks in the cold soil, and they begin feeding as soon as vegetation starts to grow. Laboratory experiments conducted during the past year have shown that newly-hatched larvae can withstand long periods of starvation when kept cool, but having once fed they succumb in a few days if no more food is available. Older larvae can withstand from two to three weeks' starvation.

During the spring of 1936, field experiments were conducted to determine the practicability of producing a starvation period for first and second instar larvae by cultivation to destroy vegetation and to delay the seeding of the new crop. Two fields of 640 acres each, which had been seeded to wheat in 1935 and were severely infested with first instar cutworms were chosen. The first of these was cultivated within a three-day period with a one-way disc. After a delay of one day, seeding was started across the direction of cultivation, which provided 12 plots of a little over 53 acres each, with the different periods of delay between cultivation and seeding varying from two to thirteen days. The second field was cultivated with a one-way disc and no seeding done for ten days after the cultivation was completed. Seeding in the first field was completed on April 29 and in the second field on May 19.

In the first of these fields, cutworms seriously injured the crop on all plots with less than a five-day delay between cultivation and seeding. The rest of the plots showed little or no damage and few cutworms were present. In the second field the cutworm loss was less than one per cent, and larvae were very scarce.

A re-study of the field histories which have been taken over a large portion of Alberta and Saskatchewan since 1921 was made with delayed seeding in mind. Only fields on farms where some cutworm damage

[1] Entomologist.

occurred and which definitely showed that a delay had or had not occurred between cultivation and seeding were considered. These fields were divided into two groups, those which showed a delayed seeding and those in which cultivation and seeding were done at the same time. Because larvae beyond the second instar can withstand longer periods of starvation than those in the first two instars, the fields showing delayed seeding were also divided into two groups. The first of these contained fields cultivated and seeded before May 15, and the second group contained those fields where the seeding at least was done after May 15. Unfortunately there is nothing in the records to indicate how many days occurred between cultivation and seeding, and the cutworm losses which occurred may have arisen from the fact that the delay was of short duration.

The results of these field history studies are as follows:

Classification	Total fields	Total acres	Acres destroyed	Per cent damage
Delayed seeding, early	31	1,869	254	13.05
Delayed seeding, late	56	2,551	741	29.04
Simultaneous cultivation and seeding	263	12,299	6,705	54.51

These records indicate that over a period of years, when seeding followed at an internal after cultivation the loss caused by pale western cutworm was greatly reduced, particularly if the cultivation and seeding were done early.

During the season of 1936 a few farm:rs were interviewed who have consistently grown crops on stubble without cutworm losses, in the midst of areas of severe cutworm infestations. These men have all followed the same practice in their fields. All stubble to be seeded is cultivated early to destroy volunteer growth, and seeded from a week to ten days after the cultivation.

While the results reported here are hardly sufficient to warrant a definite recommendation of delayed seeding as a control for pale western cutworm, they at least indicate that this practice might be of considerable benefit. One thing is certain, the damage will not be any greater if seeding is delayed, and it may be considerably lessened. More experimental work is required to determine the minimum number of days that seeding must be delayed to give a satisfactory control and what the effect of excessive rainfall in prolonging this period might be. On the other hand, excessive rainfall, in itself, will result in a reduction in the amount of damage caused by cutworms, but the minimum period of delay for average seasons must be determined.

This preliminary report is offered with the hope that investigators working on this insect in other areas will attempt similar control experiments. In this way a mass of data can be secured in a short time which will cover a great variety of conditions and seasons, and either prove or disprove the value of the control method.

SUMMARY

During the investigation for the control of the pale western cutworm, it was frequently noted that in some of the fields which had received drift soil infested with cutworm eggs, the expected outbreak of cutworm failed to develop. Upon investigating this problem in the laboratory, it was discovered that newly hatched larvae could withstand long periods of starvation when kept cool. If, however, they had fed at all, they later succumbed in a few days if no food was available. Experiments on a field scale in 1936 demonstrated that a delay of five or more days between cultivation and seeding in infested fields virtually eliminated both injury and the cutworms themselves in plots or mile-square fields. An analysis of the large number of field histories collected during the many years of study, classified in terms of at least some interval between cultivation and seeding indicated that such an interval was associated with a material reduction in the destruction of crop. The same idea was reflected in a study in 1936 of certain farms where wheat was consistently grown on stubble without cutworm loss; here, as in all cases, cultivation and seeding were separated by at least a week. While results here reported are hardly sufficient to warrant a general recommendation of delayed seeding, they are offered with the hope that others working with this insect will give the idea a test and assist in the proof or disproof of the value of the suggestion.

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Résumé

Façons culturales pouvant être utiles contre le ver gris pâle de l'Ouest, Agrotis Orthogonia Morr. H. L. Seamans, Laboratoire entomologique fédéral, Lethbridge, Alta.

Au cours des recherches faites pour trouver des moyens de combattre le ver gris pâle de l'Ouest, on a souvent noté que des champs qui avaient reçu de la terre infestée d'œufs, apportée par le vent, ne développaient pas l'invasion que cet apport d'œufs faisait prévoir. Au laboratoire où ce problème a été étudié on a découvert que les larves nouvellement écloses peuvent rester longtemps sans nourriture tant qu'elles sont tenues au frais, tandis que celles qui reçoivent de la nourriture succombent au bout de quelques jours lorsque cette nourriture est retranchée. Les recherches conduites en plein air en 1936 sur des parcelles ou des champs d'un mille carré ont fait voir qu'une attente de cinq jours au minimum entre l'ameublissement et l'ensemencement des champs infestés supprime virtuellement les dégâts, aussi bien que les vers gris eux-mêmes. L'analyse d'un grand nombre de notes sur l'histoire des champs, recueillies pendant les nombreuses années de l'enquête et classées suivant l'intervalle écoulé entre l'ameublissement et l'ensemencement, indique que cet intervalle est suivi d'une réduction considérable dans la quantité de récoltes détruite. Le même fait ressort d'une étude entreprise en 1936 sur certaines fermes où le blé avait été cultivé régulièrement sur chaume, sans souffrir des vers gris; ici comme dans tous les autres cas, un intervalle d'au moins une semaine s'était écoulé entre l'ameublissement et l'ensemencement. Les résultats signalés dans ces articles sont à peine suffisants pour que l'on puisse, en général, recommander de retarder les semailles, mais nous les offrons dans l'espoir que d'autres personnes faisant des recherches sur ces insectes mettront cette idée à l'essai et aideront à confirmer la valeur de cette pratique ou en à démontrer l'inutilité.

APPENDIX

It was from the evidence in this paper, communicated to one of us (Mr. King) by H. L. Seamans for use in correspondence with farmers, that we made our recommendation. ". . . . . . or if seeded the following practice should be followed: The land should be worked thoroughly as soon as there is considerable volunteer growth, but not before; seeding should be delayed for one week after this tillage; weather which will produce volunteer growth will hatch the cutworm eggs and cause the young cutworms to feed, and it has been found that once they have fed most of them are starved out in a few days if weed growth is destroyed, whereas grain seeded immediately after the preparatory tillage will suffer severely. It should be pointed out that this procedure has not been finally tested, but the results to date are very promising . . . . . . ", on page 11 of Bulletin 87 Saskatchewan Department of Agriculture "Grasshopper Control in Saskatchewan", 1937. This premature publication was wholly inadvertent. We desire therefore to make clear that priority and credit for this important original discovery, involving as it does an apparently new principle, belong entirely to Mr. Seamans.

THE EFFECT OF SOIL MOISTURE, HARDENING, ENDOSPERM CONDITION AND VARIETY ON THE FROST REACTION OF WHEAT, OAT AND BARLEY SEEDLINGS[1]

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The results of investigations on the effect of seedling frost injury on the subsequent growth of a series of wheat varieties have already been reported (7). Injury by frost in the seedling stage was found to delay heading and to decrease the number of fertile culms per plant. The investigations described herein are concerned with the effects of the moisture content of the soil, hardening and the condition of the endosperm on the frost reaction of wheat, oat and barley seedlings. A comparative study of varietal reactions is also described.

GENERAL METHODS

Two chambers, 9 feet by 10 feet and 6.5 feet in height, were cooled by a York refrigerating plant with overhead coils. The outer chamber was fitted with artificial lights and used for hardening, while the inner chamber was unlighted and used for freezing the plants. Temperature was controlled by a DeKhotinsky bimetalic thermo-regulator, the maximum variation within the chamber being approximately 1.5° C. Air circulation was maintained by a Sirocco suction fan and two office fans.

Unless otherwise stated, the material was grown in wooden flats containing fertile black loam soil.

In several of the experiments the plants were pre-chilled, that is, they were kept at a temperature of 0° C. for varying periods before being subjected to freezing temperatures.

A period of ten days after exposure to frost elapsed before the damage was estimated, in order to afford the plants an opportunity to recover. Four categories were used in the final classification of seedlings, as follows: class I, dead; class II, severely injured; class III, slightly injured; and class IV, apparently uninjured. The values 0.00, 0.33, 0.66 and 1.0 were assigned to individual plants falling into classes I to IV respectively. The survival index for a given lot of plants was based on the summation of the above values multiplied by 100 and divided by the number of plants involved. The survival indices, therefore, vary from 0 to 100, representing a range from complete killing to no apparent damage. The advantages of these criteria of injury are discussed by Salmon (8).

The statistical significance of observed differences was determined by the analysis of variance method described by Fisher (2). For the determination of the significance of individual mean squares, Snedecor's table of F values (9) was used.

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[1] Contribution from the Department of Field Crops, University of Alberta, Edmonton. Part of a thesis submitted to the University of Alberta in partial fulfilment of the requirements for the degree of Master of Science.

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TABLE 1.—SURVIVAL INDICES OF SPRING WHEAT VARIETIES WHEN EXPOSED TO FREEZING TEMPERATURES IN SOIL OF VARIOUS MOISTURE CONTENTS, AND WITH VARIOUS PERIODS OF PRE-CHILLING

Variety	C.A.N.*	Mean survival index											
		65% moisture holding capacity				50% moisture holding capacity				20% moisture holding capacity			
		A.	B	C	Av.	A	B	C	Av.	A	B	C	Av.
Red Bobs 222	1637	62.5	59.0	33.5	51.7	18.8	59.5	29.0	35.8	48.5	39.6	29.8	39.3
Canus	1260	39.8	66.0	42.8	49.5	30.0	68.3	34.3	44.2	15.0	41.3	21.5	25.9
Garnet	1316	27.5	62.0	25.0	38.2	19.0	54.8	23.0	32.3	41.8	27.8	19.3	29.6
Marquis	1621	46.3	39.5	30.3	38.7	24.0	51.3	32.8	36.0	10.0	33.5	23.3	22.3
Caesium 0.111	1256	30.5	65.3	14.0	36.6	24.5	59.0	11.5	31.7	17.3	49.5	12.3	26.4
Reward	1509	26.8	41.8	32.0	33.5	25.0	43.8	28.8	32.5	18.3	33.3	21.0	24.2
Average		38.9	55.6	29.6	41.4	23.6	56.1	26.6	35.4	25.2	37.5	21.2	28.0

* Canadian Accession Number.

A—Not pre-chilled. Subjected to a temperature of −12° C. for four hours.

B—Pre-chilled for four hours, temperature lowered to −12° C. and maintained at this for three hours.

C—Pre-chilled for twelve hours, subjected to a temperature of −12° C. for four hours.

SOIL MOISTURE CONTENT

Experiment 1

Methods

In this experiment six varieties of wheat were planted in soil in each of 36 flats. During the growth of the plants the moisture content was maintained at apprxoimately 50% of the moisture holding capacity of the soil. Before the flats were transferred from the greenhouse to the low temperature chambers, the soil was allowed to dry until the moisture content was reduced to about 20% of the moisture holding capacity. At this time twelve of the 36 flats were left at the 20% moisture level, and of the remaining 24, twelve were raised to 50 and twelve to 65%.

The 36 flats were treated in the following manner: Three lots, each consisting of four flats at 20, four at 50 and four at 65% moisture were subjected to freezing temperatures. The first was exposed to −12° C. for four hours without any pre-chilling; the second was pre-chilled for four hours and the temperature was then lowered to −12° C. and maintained for three hours; and the third was pre-chilled for twelve hours and then subjected to −12° C. for four hours.

Results

The results are summarized in Table 1. It is apparent that the survival indices are related to the moisture content of the soil, increases in the former being associated with increases in the latter. The relationship is, however, modified by the pre-chilling treatment, being more marked in treatments A and B then in treatment C. In other words, there is an interaction between moisture content and treatment, the significance of which is attested by the analysis of variance test (Table 2). The relative response of the seedlings to chilling in the different moisture content classes may be due to a direct effect of moisture content or to an alteration in the physiology of the plants themselves, or both. The relative survival of different varieties was not affected by soil moisture content. The statistical analysis (Table 2) offers no evidence of interaction between varieties and moisture or between varieties, moisture and treatment. There is, however, an indication of interaction between varietal reaction and treatment.

TABLE 2.—ANALYSIS OF VARIANCE OF THE SURVIVAL INDICES OF WHEAT SEEDLINGS EXPOSED TO FREEZING TEMPERATURES IN SOIL OF VARIOUS MOISTURE CONTENTS AND WITH VARIOUS PERIODS OF PRE-CHILLING

Variation due to:	Degrees of freedom	Sum of squares	Mean square	F
Varieties	5	5,025	1,005.0	4.31*
Moisture contents	2	5,979	2,989.5	12.82*
Freezing treatments	2	25,354	12,677.0	54.38*
Varieties × moisture contents	10	2,809	280.9	1.21
Varieties × freezing treatments	10	5,678	567.8	2.44†
Treatments × moisture contents	4	2,776	694.0	2.98†
Varieties × moisture contents × treatments	20	5,395	269.8	1.16
Error	162	37,770	233.1	—
Total	215	90,786	—	—

* Exceed the 1% point.
† Exceed the 5% point.

Klages (4) has shown the temperature of moist soil lags more upon exposure to freezing temperatures than does that of dry soil. It is not improbable, therefore, that the relatively high survival values secured from the 65 to 50 per cent moisture lots is due to the protective effect of water. Pre-chilling should substantially reduce this protective effect, as the long chilling process would tend to diminish the effect of the temperature lag. This supposition is borne out by the experimental results.

From the point of view of experimental technique, it is evident that in comparative varietal trials, the moisture content of the soil must be maintained at as constant a level as possible. Since it is impossible to secure absolute uniformity of soil moisture content, and since pre-chilling reduces the effects on survival of soil moisture differences, pre-chilling constitutes an essential phase of adequate technique.

Experiment 2

Methods

This experiment was designed to measure the effect on survival, or frost resistance, of soil moisture content during the growth of the seedlings. Marquis wheat was used in all tests. One-gallon glazed crocks were used as containers. Nine crocks were filled with Edmonton loam (Series I) and nine with washed sand (Series II). A complete nutrient solution was used in the sand cultures. The chemical composition of this solution, expressed as cc. of molar solution per litre, was as follows: calcium nitrate, 5; potassium nitrate, 5; magnesium sulphate, 2; and potassium acid phosphate, 1. Ferric tartrate (0.5%) was added every three days at the rate of 1 cc. per litre. Three lots, each consisting of three soil and three sand cultures, were maintained at constant moisture levels; one at 30, one at 45 and one at 60% of the moisture holding capacity of the substrate concerned. Immediately before the low temperature treatment all cultures were brought to the 60% moisture level. They were pre-chilled for twelve hours, and then exposed to $-12°$ C. for four hours.

Results

The results are summarized in Table 3. It is apparent that the moisture content of the soil during the growth of the seedlings, within reasonable limits, does not affect the survival values when moisture differences are eliminated immediately prior to exposure. In experimental trials concerned with frost reactions, soil moisture content will vary from one container to another unless special precautions are taken. It appears,

TABLE 3.—SURVIVAL INDICES OF MARQUIS WHEAT SEEDLINGS WHEN EXPOSED TO A TEMPERATURE OF $-12°$ C. FOR FOUR HOURS AT A UNIFORM MOISTURE CONTENT AFTER BEING GROWN IN SAND AND SOIL CULTURES MAINTAINED AT VARIOUS MOISTURE CONTENTS

Moisture content maintained during growth of the plants	Mean survival index		
	Sand culture	Soil culture	Average
30% of the moisture holding capacity	21.0	22.8	21.9
45% of the moisture holding capacity	20.7	18.8	19.8
60% of the moisture holding capacity	19.1	22.1	20.6

The variance due to moisture content is less than that due to error.

however, from the above results that relatively great differences in soil moisture content during growth do not affect the frost reaction and, consequently, the maintenance of uniform moisture content is unnecessary.

HARDENING

Methods

Eight spring wheat varieties, grown in flats, were pre-chilled for 36, 24, 12 and 0 hours, and then exposed to $-10°$ C. for four hours. Each variety was replicated six times within each hardening treatment.

Results

The data are summarized in Table 4. Pre-chilling resulted in a marked increase in frost resistance, and the 12, 24 and 36 hour periods were equally effective in this respect. The ranking of the varieties in the different treatments, while not identical, does not differ significantly. Similar results were obtained by Peltier and Kiesselbach (6).

In addition to increasing the frost resistance of the material, pre-chilling reduced the coefficient of variability. The amount of reduction was similar in the 12, 24 and 36 hour series. The maintenance of uniform temperature conditions throughout the freezing chamber is a problem of considerable magnitude. The difficulty is undoubtedly aggravated by the relatively rapid radiation of heat from unchilled flats by which air currents are set up. Consequently, in tests of unchilled material, temperature conditions are likely to be less uniform and the variability of survival correspondingly large. With pre-chilled material the air currents are less violent and conditions more uniform, resulting, without a doubt, in more accurate comparisons.

TABLE 4.—SURVIVAL INDICES OF SPRING WHEAT VARIETIES PRE-CHILLED FOR VARIOUS PERIODS OF TIME AND THEN EXPOSED TO A TEMPERATURE OF $-10°$ C. FOR FOUR HOURS

Variety	Survival index				
	Pre-chilled 36 hours	Pre-chilled 24 hours	Pre-chilled 12 hours	Pre-chilled 0 hours	Mean
Ceres	67.8	68.3	75.8	19.8	57.9
D.C. I-28-65	64.7	73.3	67.2	26.3	57.9
Red Bobs 222	68.3	71.8	65.5	24.3	57.5
Reliance	63.8	63.7	73.2	23.0	55.9
Canus	66.2	70.2	60.2	17.5	53.5
Garnet	50.3	52.3	63.5	9.8	44.0
Marquis	61.7	53.8	42.7	9.3	41.9
Reward	54.0	48.7	35.0	16.8	38.6
Mean	62.1	62.8	60.4	18.4	50.9

F value for treatment variance divided by error variance = 54.26. 1% point = 3.90
Variety × treatment variance was less than error variance.

CONDITION OF THE SEED

Methods

In testing a series of varieties for frost resistance, it is not always possible to secure equally good seed of all varieties. This experiment was designed to test the effect, if any, of frost damage and artificial mutilation of seed on the frost resistance of seedlings grown therefrom. Plots of Marquis wheat were planted at several different points on the University

farm in 1934. The plots were not all equally damaged by the early autumn frost that year, and so it was possible to select three samples as follows: apparently undamaged, lightly frozen and severely frozen. A fourth sample was prepared by the removal of a part of the endosperm.

The weight per 1000 kernels of the four lots is recorded in Table 5. Eight-replicate plantings of each lot were made on Edmonton black soil and on relatively infertile podsolic loam secured from Fallis, giving a total of 16 plantings of each type of seed. When the seedlings reached the two-leaf stage they were pre-chilled for 12 hours and then exposed to −10° C. for four hours.

Results

The survival indices (Table 5) of the different lots do not differ significantly. It is apparent, therefore that the frost reaction of Marquis wheat seedlings is not affected by frost damage, even though the seed may be severely shrunken, or by mutilation involving the removal of a major portion of the endosperm.

TABLE 5.—THE EFFECT OF DIFFERENCES IN SOIL TYPE AND ENDOSPERM CONDITIONS ON THE SURVIVAL INDICES OF MARQUIS WHEAT SEEDLINGS EXPOSED TO A TEMPERATURE OF −10° C. FOR FOUR HOURS

Condition of the seed used	Weight per 1000 kernels of the seed used	Mean survival index		
		Black soil	Gray soil	Average
Normal	35.5	77	66	72
Endosperm partially removed	24.7	73	67	70
Slightly frozen	31.2	79	66	73
Severely frozen	22.7	68	66	67

The variance due to seed treatments is less than that due to error.

THE REACTION OF WHEAT, OAT AND BARLEY VARIETIES TO FREEZING TEMPERATURES

Methods

Two 6-replicate tests of 32 wheat varieties were conducted; the first in August 1935, and the second in March 1936. In each replicate approximately 20 plants of each variety were grown. The first test involved an exposure to −8° C. for four hours, and the second to −11° C. for the same period. In both tests the plants were pre-chilled for 12 hours before exposure. Two similar tests of 29 varieties of barley were conducted in December, 1935 and February, 1936. A single 4-replicate test of 15 oat varieties was made. After pre-chilling for 12 hours the seedlings were exposed to −10° C. for four hours.

Results

The data obtained from the wheat, oat and barley trials are summarized in Tables 6, 7, and 8 respectively.

The majority of the relatively highly resistant wheat varieties are selections from crosses between spring and winter varieties. There is an indication of a relationship between lateness of maturity and frost resistance. Relatively late varieties such as Canus, Red Fife, Reliance, Huron and Ceres, are moderately resistant, while early varieties such as

Reward, Khogoh, Garnet and Ruby are relatively susceptible. The varieties do not rank in the same order in −8° C. and −11° C. tests. However, since the statistical analysis failed to reveal a significant interaction between variety and treatment, the observed differential response of varieties to the treatments may be attributed, largely at least, to experimental error.

TABLE 6.—SURVIVAL INDICES OF WHEAT VARIETIES AFTER EXPOSURE TO TEMPERATURES OF −8° C. AND −11° C.

Variety	N.S.N.*	C.A.N.	Mean survival index		
			At −8° C.	At −11° C.	Average
Marquis × Kanred	I-28-139	—	93.3	68.5	80.9
Ridit × Ceres	I-33-36	—	93.2	68.6	80.9
Canus	I-28-114	1260	89.2	72.2	80.7
Milturum 0.321	I-28-14	1415	80.0	74.8	77.4
Marquis × Kanred	I-28-137	—	93.2	60.3	76.8
Milturum 0.274	I-28-25	1628	84.7	68.3	76.5
Marquillo × (Marq.-Kan.)	I-28-65	—	84.7	67.1	75.9
Red Fife	I-0-19	1515	83.5	64.7	74.1
Marquis × Kanred	I-28-39	—	78.8	68.5	73.7
Reliance	I-29-4	1498	84.8	62.4	73.6
Ceres	I-25-1	1263	83.2	64.0	73.6
Marquillo × (Marq.-Kan.)	I-28-60	—	88.7	56.1	72.4
Preston	I-30-3	1635	82.2	61.5	71.9
Progress	I-29-7	1590	79.7	62.5	71.1
Hussar × Hard Federation	I-32-42	—	85.0	56.4	70.7
Marquis × Kanred	I-28-138	—	79.8	60.2	70.0
Marquillo × (Marq.-Kan.)	I-28-64	—	82.7	56.9	69.8
Caesium 0.111	I-28-20	1256	82.3	56.0	69.2
Reliance × Hope	I-31-10	—	75.8	62.0	68.9
Red Bobs 222	I-0-18	1637	71.0	66.5	68.8
Early Triumph	I-25-12	1291	85.0	50.5	67.8
Renfrew	I-0-20	1514	75.5	58.9	67.2
Marquis	I-0-9	1621	73.0	58.2	65.6
Kitchener	I-0-5	1363	81.5	49.5	65.5
Reward	I-25-21	1509	68.5	56.9	62.7
Hope × Ceres	I-31-12	—	68.7	55.8	62.3
Huron	I-0-4	1344	75.7	48.0	61.9
H42 × Marquis	I-32-29	—	66.8	52.8	59.8
Hope	I-29-9	1615	73.3	44.7	59.0
Khogoh	I-34-14	—	66.7	49.3	58.0
Garnet	I-25-13	1316	69.7	46.2	58.0
Ruby	S-22-42	1511	59.5	36.0	47.8

* University of Alberta Nursery Stock Number.
F value for variety variance divided by error variance = 3.68. 1% point = 1.80.
Variety × temperature variance was less than error variance.
The minimum significant difference between varietal averages is 11.3 units.

In the oat variety test (Table 7), Winter Turf and a culture of *Avena brevis* exhibited the highest degree of resistance. The common late and mid-season varieties Victory, Leader and Banner proved moderately resistant, while early varieties such as Nidar, Alaska, Gopher and Legacy were decidedly susceptible.

The statistical analysis of the test of barley varieties revealed a significant interaction between variety and treatment (−8° C. and −11° C.). The variance of variety means did not significantly exceed that of variety

TABLE 7.—SURVIVAL INDICES OF OAT VARIETIES EXPOSED TO A TEMPERATURE OF −10° C.

Variety	C.A.N.	Mean survival index	Variety	C.A.N.	Mean survival index
Winter turf	291	53.0	White Cross	601	22.8
A. brevis	—	48.8	Liberty	504	21.0
Black Tartarian	188	36.5	O.A.C. 144	39	16.8
Red Rustproof	514	32.7	Nidar	643	15.5
Leader	199	31.3	Alaska	458	13.0
Victory	518	31.0	Gopher	14	10.5
Black Algerian	174	29.0	Legacy	460	10.3
Banner	62	27.0			

The minimum significant difference between survival indices is 18.0 units.
F value for variety variance divided by error variance = 4.16. 1% point = 2.60.

and treatment interaction and, consequently, significant differences between variety means were not established. Separate analyses based on the data of individual treatments, however, reveal highly significant differences between varietal averages within treatments. At −11° C. the early maturing variety Pannier was moderately resistant but the other early maturing varieties, notably Olli, Lapland and Ottawa 1014, were quite susceptible. Among the varieties responding differently to the two temperatures, Regal, Colsess, Pannier and Manchurian, are outstanding. An examination of the data in Table 8 reveals the fact that Regal, for example, exhibited moderately high resistance at −8° C., but was susceptible at −11° C. Manchurian, on the other hand, was susceptible at both temperatures.

The relatively weak correlation between the survival indices of barley

TABLE 8.—MEAN SURVIVAL INDICES OF BARLEY VARIETIES AFTER EXPOSURE TO TEMPERATURES OF −8° C. AND −11° C.

Variety	C.A.N.	Mean survival index	
		At −8° C.	At −11° C.
Sacremento	744	74.0	55.2
Atlas	702	68.7	44.3
Pannier	1042	44.5	36.9
Trebi	753	66.7	36.0
Eureka	773	57.0	35.6
O.A.C. 21	734	64.0	34.6
Vaughn	1090	52.3	32.7
O.A.C. 21	1086	71.0	32.4
Nobark	1022	47.8	28.2
Himalayan	765	40.0	28.2
Glabron	718	68.0	27.0
Success	783	64.2	25.8
Peatland	722	62.0	24.4
Regal	742	72.2	23.3
Colsess	772	72.0	22.5
Spartan	860	63.2	22.2
Comfort	712	50.2	20.8
Manchurian	724	27.3	20.8
Velvet	755	53.5	19.9
Bearer	704	30.2	19.4
Gordon	833	45.3	17.3
Ottawa No. 1014	1105	53.3	17.0
Lapland	877	44.8	16.6
Gold	829	59.2	15.5
Olli	739	49.5	13.9
Hannchen	837	60.3	13.7
Newal	1089	27.5	11.1
Canadian Thorpe	816	49.0	10.3
Sanalta	1088	50.3	8.7

The F value for variety variance divided by variety × temperature variance is 1.11. 5% point = 1.88.

The F value for variety variance divided by error variance = 8.51. 1% point = 1.85.

The minimum significant difference between any two varietal averages at −8° C. is 14.2, and at −11° C. 14.1 units.

varieties tested at two different temperatures emphasizes the importance of selecting a treatment suited to a particular purpose. If selection for frost resistance is the chief aim, a relatively severe treatment should be employed. If, on the other hand, the purpose is to rank a series of varieties or strains according to frost resistance, a treatment which will give the widest possible range should be selected. The necessity of preliminary trials is obvious.

DISCUSSION

The fact that the early varieties of wheat, oats and barley were found to be generally susceptible to frost injury suggests that a relationship may exist between these characters. In the experiments conducted, all of the varieties in a given test were seeded at one time and all the resulting plants were exposed to freezing temperatures at a given number of days after seeding. It is, therefore, possible that the early varieties were actually at a later stage of development, when exposed to the freezing temperatures, than were the later varieties. Then, if plants become more susceptible at later stages of development, the differences in survival would be at least partly due to this cause rather than to the inherent potentialities of the varieties concerned. However, at such an early stage of development, it is difficult to believe that the varieties differed to an extent sufficient to account for the differences noted. Certainly any differences present were not noticeable to the eye.

If we assume that cold susceptibility is an inherent property of these early varieties, the suggestion at once presents itself as to the possibility of an association between factors for earliness and factors for cold susceptibility. In this connection, it might be noted that Neatby (5) has shown that winter habit in barley is in reality the expression of an accumulated series of late growth factors. There would appear to be some grounds for assuming that winter habit is associated with cold resistance.

As earliness is, and cold susceptibility probably is, controlled by several genetical factors, any association between these characters would probably be due to the multiple effect of single genes rather than to a linkage between genes for earliness and those for cold susceptibility.

Any degree of association between these characters would tend to the production of early cold susceptible varieties and late cold resistant varieties, as no conscious selection has been made for cold resistance among the hybrid populations.

From the standpoint of crop improvement, this whole question deserves attention from the plant breeder. At present, considerable work is being done on the production of new early varieties of cereals. Even if it is granted that no association exists between earliness and cold susceptibility, if cold susceptible varieties are used as parental stocks, and no attention is paid to the cold resistance of the progeny, then there is a danger of obtaining varieties as susceptible, or more so, than those at present in use. Since these new varieties are designed for areas where frost injury is most likely to be a serious limiting factor in crop production, and since it appears that seedling frost damage seriously delays the maturity of the plants so injured (7), the danger involved appears to be sufficiently serious to warrant some attention. The knowledge of the approximate mode of the inheritance

of cold resistance in cereal crops, and more particularly of the associations, if any, between this character and any other characters, would therefore be of considerable value to plant breeders who are developing crops for northern regions.

SUMMARY

Hardened and unhardened wheat plants were grown in soil maintained at 50%—and subjected to freezing temperatures in soil maintained at 20, 50 and 65%—of its moisture holding capacity. Survival was directly related to moisture content. The relative varietal reaction was the same at each of the different moisture contents. The survival of plants hardened for 12 hours was influenced less by variations in moisture content than was that of unhardened plants. Plants were grown in sand and in soil maintained at 30, 45 and 60%—and exposed to −12° C. for four hours at 60%—of the moisture holding capacity of the substrate. The survival values of the plants were not significantly different.

Wheat varieties were hardened for 36, 24, 12 and 0 hours at 0° C. and then exposed to −10° C. for four hours. Hardening markedly increased the frost resistance of the varieties concerned. The relative varietal reaction was the same in each hardening treatment.

The survival indices of plants grown from normal, slightly frozen, severely frozen, and seed from which a portion of the endosperm had been removed, were found to be essentially similar.

Wheat varieties were found to differ distinctly in their reaction to freezing temperatures in the seedling stage. Similar differences were noted among oat and among barley varieties. The relative reactions of certain barley varieties were found to be dependent upon the freezing temperatures to which they were exposed. Such differential responses were not found among the wheat varieties tested. Attention is drawn to the fact that the early maturing varieties of cereals tested in these experiments were found to be relatively susceptible to frost injury in the seedling stage.

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Résumé

L'effet de l'humidité du sol, de l'endurcissement, de l'état de l'endosperme et de la variété sur le comportement à la gelée des plantules de blé, d'avoine et d'orge. A. W. Platt, Université de l'Alberta, Edmonton.

Des plants de blé endurcis et non endurcis ont été cultivés dans un sol maintenu à 50% de sa capacité d'absorption d'humidité—et soumis à des températures de congélation dans un sol maintenu à 20%, 50% et 55% de sa capacité d'absorption. On a constaté que le comportement relatif des variétés est le même, à chacune des trois proportions d'humidité. La survivance des plants endurcis pendant 12 heures est moins affectée par les variations dans la proportion d'humidité que celle des plants non endurcis. Des plants ont été cultivés dans du sable et dans de la terre, maintenus à 30, 45 et 60% de leur capacité d'absorption d'humidité—et exposés à $-12°$ C. pendant quatre heures à 60% de la capacité d'absorption de la couche inférieure. Les chiffres indiquant les valeurs de survivance des plants n'étaient pas très différents. Des variétés de blé ont été endurcies pendant 36, 24, 12 et 0 heures à 0° C. puis exposées à $-10°$ C. pendant quatre heures. La résistance à la gelée des variétés en question a été grandement augmentée par l'endurcissement. Le comportement relatif des variétés était le même dans chaque traitement d'endurcissement. Les indices de survivance des plants provenant de semence normalement, légèrement et fortement gelée et dépouillée d'une partie de leur endosperme étaient essentiellement semblables. On a constaté que les variétés de blé diffèrent beaucoup l'une de l'autre par la façon dont elles se comportent sous des températures de congélation lorsqu'elles sont à l'état de plantules. Des différences semblables ont été notées dans l'avoine et parmi les variétés d'orge. On a constaté que le comportement relatif de certaines variétés d'orge dépend des températures de congélation auxquelles elles sont exposées, mais on n'a pas constaté les mêmes différences dans le comportement des variétés de blé à l'essai. On appelle l'attention sur le fait que les variétés hâtives de céréales, essayées dans ces expériences, ont été relativement sensibles à la gelée dans la phase de la plantule.

SWEET CLOVER STUDIES ON HABIT OF GROWTH, SEED PIGMENTATION AND PERMEABILITY OF THE SEED COAT[1]

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INTRODUCTION

Sweet clover, notwithstanding its wide adaptation to soil and climatic conditions and its increasing importance as a cultivated hay and pasture crop in the prairie provinces of Canada and in the adjacent states of the United States, possesses certain undesirable features which have tended to restrict its use agriculturally. Among these may be mentioned the coarseness of the stems, which detracts from its value as a hay crop, and the impermeability of the seed coat, which necessitates scarification of the seed before it is sown. The production of improved varieties has engaged the attention of plant breeders during recent years. Much work has been done and considerable progress has been made, especially in the production of finer stemmed, more leafy types.

Up to the present time improvement has been accomplished largely through selection from the numerous naturally occurring types. The possibility of combining, through hybridization, the desirable characteristics of two or more varieties has received little attention. Nevertheless, much valuable data, regarding the breeding behaviour of characters of economic interest, have been obtained.

The data presented herein deal with the mode of inheritance of the mottled seed character and of the dwarf branching habit of growth and with the occurrence, cause and nature of the permeable or soft coated seeds in white blossom sweet clover, *Melilotus alba* Desr.

The colour of well matured seed of *M. alba* is, in most cases, golden yellow. Recent investigations have shown, however, that mottled seeds, in which the seed coats are marked with distinct flecks or splashes of reddish or purplish pigment, are sometimes found among the seeds of this species. In addition, certain new varieties and strains of *M. alba* have been produced in which the seed coats are usually mottled. In the past, mottled seeds have been regarded as characteristic of certain yellow flowered species, particularly *M. officinalis* (L) Desr., and the presence of seeds carrying markings of reddish or purplish pigment in samples of common white blossom biennial sweet clover seed has been regarded as evidence of a mixture of the yellow blossom species. At present, according to the Seed's Act, as much as 2% of mottled seed is permitted in seed from certain varieties of *M. alba* in Canada.

The mottled condition in sweet clover does not take the form of distinct patterns such as are sometimes found in seeds of certain other legume

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species. The ground colour of the seeds is normally golden yellow or greenish yellow and the reddish or purplish pigment is distributed at random in flecks or splashes of various shapes and sizes. All gradations of mottling are found, from one or two mere specks which can be located only through careful examination under a lens, to seeds in which the ground colour is almost obscured by the pigment.

The term "dwarf branching" has been used to describe the habit of growth peculiar to the "Alpha" variety of *M. alba*. These plants differ from the common type of white blossom biennial sweet clover in several important characteristics. They are usually not more than 50 to 75% as tall as the common type and produce numerous stems from a much branched crown, as compared with the relatively few stems of common type plants. The stems of the dwarf branching type plants too are comparatively small in diameter and the whole plant produces a relatively high percentage of leaf. In most other respects they are indistinguishable from the common type plants of *M. alba*. These two types hybridize readily.

The occurrence of "hard" seeds in many leguminous plants is of both scientific and commercial interest. These "hard" seeds have been defined as seeds whose coats are impermeable to water at temperatures favourable for germination. In sweet clover this impermeable condition is due to certain peculiarities in the structure of the seed coats. In Western Canada the percentage of permeable seed in our cultivated sweet clovers varies from 0 to 8% with an average of less than 1%.

Various treatments are at present employed for the purpose of rendering sweet clover seeds permeable before they are sown. The treatment most commonly used is scarification of the seed coat, through the use of various devices. The treatment consists usually in blowing the seeds with considerable force against a surface covered with emery or sandpaper. When properly done, such treatment causes most of the seeds to become permeable. Machines necessary for this type of work are expensive and growers who wish to produce their own seed are forced to improvise some less efficient method of treating their seeds. Probably no other single factor has tended to restrict the use of sweet clover agriculturally to so great an extent as has the necessity for scarification of the seed.

LITERATURE REVIEW

Mottled Seed

Many workers have reported the results of investigations into the mode of inheritance of the mottled condition of the seed coats in various legumes. For the most part, these studies have been made with field beans or with soybeans. Emerson (6) crossed self coloured beans with certain white-seeded varieties and found that the F_1 plants produced mottled seeds. He found two distinct sorts of behaviour of mottling in beans, and the data obtained indicated that two distinct factors for mottling were involved. The factor M produced constant mottling and the factor X inconstant mottling, which is visible only in the heterozygous condition. The relation between M and X was not determined. Both genes were effective only in the presence of P, the basic factor for pigmentation. Shaw and Norton (22) crossed pigmented with non-pigmented garden beans and obtained

segregations approaching 3:1—pigmented to non-pigmented—in the F_2. Crosses between mottled and white-seeded varieties gave mottled-seeded F_1 plants and mottled, self-coloured and white in the proportions of 9:3:4 in the F_2. Sax and McPhee (20) studied the inheritance of pigmentation in bean seeds and concluded that it was controlled by a factor P, the recessive of which results in white. They attributed the mottled condition of the seed coat to the presence of two factors in the same linkage group, both of which are necessary to produce the mottled condition. Owen and Burgess (18) investigated the influence of environmental factors on pigment patterns in varieties of common beans. They concluded that the most important factors affecting the development of seed coat pigments are probably those related to nourishment of the plant. More pigment was produced in the seeds of plants grown on rich than on poor soil. They point out that classification of the material is subject to error due to the wide variations caused by different physiological conditions.

Kristofferson (15) studied the inheritance of mottling in field beans in which brown and violet colours were involved in addition to the yellowish white normal colour. Factors for these colours he designates as B, D and P respectively. Two additional factors for mottling, Y and Z, were also found. B and D + Y and Z gave a bean mottled in violet and brown on a yellowish white background. This type of bean segregated in the F_2 to give 9:3:3:1 mottled, brown, violet and yellowish white respectively.

Hollowell (8) studied the effect of various factors on the mottling of soybean seeds. He found that the development of pigment in the seed coat of mottled beans was affected by soil but not by climatic conditions. More mottling was evident among the seeds of plants grown in rich soils than in soils lacking in fertility. Woodworth and Cole (24) found marked variation in the type of mottling exhibited by seeds from different pods on the same plant and also between different plants within a pure line. They conclude that mottling is markedly affected by physiological factors. Owen (18) found mottling in soybean seeds to be due, in some cases, to environmental factors. Difficulty was encountered in distinguishing the influence of heredity from that of environment. He concluded that where heredity alone is the controlling factor other factors conducive to pigment formation are obscured. Black and brown beans have such factors and breed true. He found that rich soils particularly were conducive to mottling in soybean seeds and that the tendency to produce mottling was more pronounced in varieties with tawny pubescence than in those with gray pubescence. Dimmock (4) concluded that certain varieties of soybeans are more resistant to mottling than others, but that the wide variation in degree of mottling within a variety is due to environmental influences.

Dwarf Branching Character

Three workers have reported the occurrence of dwarf-branching types in populations of normal *M. alba*. Kirk (9) describes certain aberrant forms, selected from "Arctic" sweet clover, as having numerous fine stems and branching at the crown like alfalfa. Elders (5) reports the finding of a dwarf, bushy, white flowered plant possessing numerous fine stems, in a field of common *M. alba*. Suvorov (23) has reported the finding of lucerne-like forms of biennial white blossom sweet clover growing among wild,

common type plants of *M. alba* in the North Caucasus area of Russia. He describes them as having greatly increased tillering capacity, thin stems, low coumarin content and as tending to ripen seed uniformly, throughout the plant. In other respects the plants were indistinguishable from the common white blossom, biennial types.

The inheritance of the dwarf branching type of plant was studied by Elders (5), who found the dwarf-bunch type of growth to be inherited as a simple recessive to the common type plant. Hybrids from natural crosses between the dwarf and common types gave segregations in the F_2 progenies closely approaching 3: 1 ratios. The common type of growth was dominant. Kirk (10) describes the segregation in progenies from ten aberrant plants. They all segregated into "aberrant" dwarf branching types and common sweet clover types. Kirk (13) studied the inheritance of the dwarf branching type of growth in crosses between Alpha and Arctic. He found the common type of growth to be completely dominant over the dwarf branching type. Segregations obtained from forty-three F_2 families gave a close fit to a 3: 1 ratio. Clarke (2) found that crosses, between dwarf branching types and common type of white blossom biennial sweet clover, gave segregations in the F_2, which showed reasonably close fits, in most cases, to 3: 1 ratios. Two exceptional cases were explained on the basis of linkage complications.

Hard Seeds

Several workers have reported the results of detailed studies of the structure of the seed-coats of various plant species, particularly legumes, in relation to permeability. Investigations, dealing with the development of strains or varieties with permeable seeds from cultivated species which normally produce hard seeds, have been reported in only a few instances. Malphigi, cited by Coe and Martin (3), gave the first account of the structure of the seed coats of legumes. Pammel (19) made an extensive study of legume seeds and reported the seed coat to consist of three layers, the outer layer of Malphigian cells, the Osteosclerid layer and the inner layer of nutrient cells. Coe and Martin (3) compared the structure of the seed coats of permeable and impermeable seeds of *M. alba* and *M. officinalis*. They concluded that the absorption of water by the seeds was not prevented by the cone shaped structures of the Malphigian cells but by the "light line." They found that the principle difference in the coats of permeable and impermeable seeds was in the thickness of the cell walls. In the Malphigian cells of the permeable seeds the cell cavities extended across the light line and formed passageways through which the moisture entered, while in those of the impermeable seeds the main cavities, due to greater thickening of the cell walls, stopped farther below the light line. The light line in the impermeable seeds was usually broader than in the permeable seeds. Lute (16), working with alfalfa, concluded that the region in the seed coat commonly known as the "light line" is an optical illusion and found the absorption of water into the seed to be governed by the Malphigian cells. She failed to find any differences between the structures of the coats of permeable and impermeable seeds. Hamly (7) describes the impermeable region of the seed coats of *M. alba* as being formed by the layer of tightly appressed suberin caps of the Malphigian

cells. He found the permeability of naturally "soft" seeds to be due to the opening of a cleft at the strophiole. In no case did he find the entire seed-coat, or part of the seed-coat other than the strophiole, responsible for permeability in naturally permeable seeds.

Sengbush and Loschakowa (21) produced, through systematic selection, "soft coated" lines of *Lupinus luteus*. They describe "soft coated" seeds as those which never become impermeable during unfavourable weather conditions. Normal seeds of *L. luteus* become "hard coated" in dry or warm storage. The method followed was to first dry the seeds of single plant selections for three months at temperatures ranging from 20° C. to 25° C. Following this, the seeds were soaked in water for from two to three hours and then examined for permeability. Only a very small percentage of the plants tested were found to produce permeable seeds. Investigations into the nature of the permeability showed that the entire seed coat, and not any particular parts of it, was responsible for the permeable condition. Zenari (25) selected for "soft coatedness" in the seeds of several plant genera including Leguminoseae. He found that three generations of continuous selection did not noticeably influence the percentage of hard seeds in any of the species worked with. He concluded that the hard seed character is irregular and cannot be considered hereditary. He found impermeability to be strictly related to the degree of maturation of the seed and to be greatly influenced by climate and soil. Certain bacteria and fungi were found in some instances to alter the tegument of the seeds and tended to make them permeable.

EXPERIMENTAL METHODS AND MATERIALS

Mottled Seed Investigations

A description of the materials and methods used in each of the three studies will be given separately.

The material used in the mottled seed investigations was obtained from two distinct sources. Line S-30-58 originated from a cross between the varieties Alpha 5 and Arctic. The origin of Alpha 5 is given by Kirk (11) and that of Arctic by Champlin and Kirk (1). Kirk and Stevenson (12) describe Alpha 5 as a type of white blossom biennial sweet clover characterized by comparatively numerous and short fine leafy branching stems. The seeds are greenish yellow and are distinctly marked with flecks or splashes of reddish or purplish pigment.

The Arctic variety is a hardy, semi-dwarf, relatively fine stemmed type of white blossom biennial sweet clover. Investigations conducted with this variety by Kirk and Stevenson (12) showed that while approximately 99% of the plants produced "clear" or non-mottled seeds, a few plants occurred which produced seeds that were strongly flecked with reddish or purplish pigment.

The Alpha plant, used as the female parent in this cross, was found to breed true for the mottled condition of the seed-coat. The Arctic male parent was likewise found to be homozygous for the clear or non-mottled condition of the seed-coat.

The F_1 plant from this cross was grown to maturity in a greenhouse which was screened to prevent the entrance of insects. The flowers were

manipulated in order to insure pollination and selfed seeds were obtained. An F_2 progeny was sown in flats in the greenhouse and the plants were later transplanted to the field nursery. Self-fertilized seeds, as well as open pollinated seeds, were obtained from most of the F_2 plants. The seeds from each F_2 plant were classified for the presence or absence of reddish or purplish markings on the seed-coat. F_3 progenies which were also handled in the manner described above were grown from each F_2 plant from which self-fertilized seeds were obtained.

The second lot of material used in the mottled seed study arose from a single plant, No. S-26-3-5, which was taken, along with several other plants, to the greenhouse in the fall of 1928. This plant was later shown to be of hybrid origin and produced progenies which segregated for mottled and non-mottled seeds. The hybrid plant was grown to maturity in the greenhouse and self-fertilized seeds were obtained from it. F_2 and F_3 progenies were grown in the field nurseries and were handled after the manner described for the S-30-58 material. Additional F_2 progeny plants and F_3 progenies were grown to maturity in the greenhouse.

Self-fertilized seeds from all plants of both S-30-58 and S-26-3-5, grown in the greenhouse, were obtained by manipulating the individual flowers with toothpicks. This consisted of gathering pollen on the flat end of the toothpick and applying it to the stigma. A new toothpick was used for each plant so as to eliminate danger of cross pollination from that source.

Self-pollination of the plants in the field nurseries was insured through the use of various types of bags and of cotton cages. Bags made from tiffany, approximately 14 inches long by 9 inches wide and fitted with a cord at the open end, were used extensively. Parchment bags, heavy brown paper bags and glassine bags were also used. Large cotton selfing cages, as described by Kirk (14), were used on a number of plants.

Bagging or caging was done, as far as possible, before any of the flowers opened. Open flowers were carefully removed, where such occurred, prior to setting the cages or selfing bags in place.

In the use of tiffany bags care was taken to enclose only two or three branches in each and to place the bags in such a manner as to permit considerable terminal growth of the enclosed branches, after bagging was done, without causing the flowers to be pushed tightly against the inside of the netting from which the bags were made. This precaution prevented possible cross pollination by insects outside the bags. Self-pollination of the bagged flowers was insured by gently rolling the bags between the palms of the two hands from time to time as new flowers opened.

Fumigation of the caged plants was necessary about twice during the season, in order to prevent serious injury from aphids. These insects caused no injury to the flowers enclosed in the tiffany bags.

Before harvest time two envelopes were prepared for each plant within each row of the nursery. Seeds from open pollinated flowers were collected into one envelope and the other was reserved for self-fertilized seed. Seeds from each plant, other than those enclosed in the selfing bags, were stripped from the racemes by hand. Only well matured pods were harvested. The self-fertilized seed produced under cages was also harvested in this manner. The seed produced in the selfing bags was harvested by severing the

branches just below the bag. Each bag was first carefully labelled with row and plant number. These harvested bags were stored and the seeds were threshed and classified during the winter months.

Both open-fertilized and selfed seeds were classified for the first progenies grown, but as no marked differences could be found, later classifications were confined largely to the open seed. In cases where the classification was doubtful the selfed seeds were also examined. Classification was accomplished by removing the hulls and spreading the seeds in a large-sized petri dish. These were examined in suitable light under a low power binocular. Heavily mottled seeds could be identified readily, in many cases, without the aid of the binoculars, but lightly flecked seeds could be classified only through the use of a lens and then often with great difficulty.

Chemical tests were made to determine the nature of the pigment present in the seeds and the seeds were sectioned and studied histologically to determine the location of the pigment in the seed coats.

Dwarf Branching Habit of Growth

The material used in this study was the same as was used in the study of the mottled condition of the seed. Plant S-30-58, which resulted from a cross between Alpha 5 and Arctic, produced progenies which segregated for the dwarf branching and common types of growth. Plant S-26-3-5 also proved to be heterozygous for plant type as well as for seed coat characters. Hence the progenies which were classified for mottled and non-mottled seeds were also classified with respect to type of plant.

Classification for plant type was made first in the seeding stage, before the plants were transplanted to the field nurseries. In most cases the dwarf branching character was clea·ly expressed at this stage but certain plants did not show the tendency to branch at the crown until later in the first year's growth, as described by Kirk (13). The plants were classified in the field in early fall of the first year and again in early summer of the second year. Except for a few individuals, there was no difficulty experienced in identifying the two types of plants.

"Hard" Seed Study

The original material used in this work was a number of single plant selections made in the fall of 1931. Selections were taken from bulk fields and from increase plots of various varieties and strains of *M. alba* and from a number of inbred lines of the Arctic variety. Seeds were harvested from the single plant selections by hand. The seeds were stripped from the racemes and stored in paper envelopes. Only bright appearing, well matured pods were taken.

The seeds from each selection were tested for permeability of the seed coat. This was done by determining the percentage germination in each case. The handling of the seeds from harvesting to germination was done carefully so as to avoid scratching the seed coats or otherwise rendering naturally hard seeds permeable. The pods were removed by rubbing the seeds lightly on a rubber mat. The light material was then blown away, and sound, bright, well matured seeds were counted out for testing. Germination tests were made in duplicate. Fifty seeds were used for each test. On account of the large number of selections, it was necessary to

devise some method which would permit many samples to be tested simultaneously in limited space. The procedure finally adopted consisted of the use of small glass vials. Fifty seeds were placed in each vial, after which a small amount of water was added and the vial rotated gently so that the moist seeds adhered to the sides. The vials were then stoppered loosely and placed in special cases which accommodated one hundred tests. Each vial lay in a groove in a sloping position so that the stoppered end was highest. The vials were placed in a dark chamber and counts were taken at the end of fourteen days. The seeds in the vials remained moist throughout the test without further addition of water. This method of testing was checked by comparing the results with those obtained by the official seed testing laboratory, Dominion Seed Branch, Saskatoon. The results of the two tests agreed within reasonable limits of variation. More than twelve thousand selections were tested in this manner.

Progenies were grown from selections which showed more than 10% permeability, as well as from several selections in which all of the seeds were impermeable. These progenies were seeded in flats in the greenhouse and later transplanted to the field nurseries in the usual manner. Self-fertilized seeds were obtained from the progeny plants by the use of selfing bags. Open-pollinated seeds were also harvested. The seeds from these first generation progeny plants were again classified for permeability on the basis of the germination test and second generation inbred progenies were grown. Selections were made from the second generation progeny plants on the same basis; hence there has been continuous selection of the most permeable and least permeable individuals, within inbred lines.

The seed coats of both permeable and impermeable selections were studied histologically to determine whether any differences in structure might exist. The osmic acid test, as described by Hamly (7), was also used to determine the location of the permeable areas in the seed coats.

EXPERIMENTAL RESULTS

Mottled Seed Study

Hybrid plant number S-30-58, which resulted from a cross between Alpha 5 and Arctic, produced white flowers and seeds which were distinctly marked with reddish or purplish pigment. The seeds obtained from this plant were all self-fertilized.

A progeny of 148 F_2 plants was grown and self-fertilized seeds were obtained from 108 of these. All of the F_2 plants were caged or bagged but the bags on several of the plants were destroyed before seed setting was completed. Open-pollinated seeds were obtained from each of the 148 F_2 plants and these were examined for the presence or absence of mottling; 117 of the plants produced mottled seeds and 31 produced seeds which were clear or non-mottled.

F_3 progenies of approximately 30 plants each were grown from 108 of the F_2 plants; 26 of these progenies consisted of only mottled seeded plants; 63 segregated for both mottled and clear seeded individuals; and 19 consisted of clear seeded plants only.

On the basis of data obtained from the F_3 progenies, it was found that three F_2 plants had been wrongly classified. The corrected F_2 classification was then 114 mottled to 34 clear seeded plants.

The F_2 data were tested for goodness of fit to a 3:1 ratio by means of the X^2 distribution and a X^2 value of .3437 was obtained. For one degree of freedom the corresponding P value is between .50 and .70. Hence a deviation as great as that obtained would be expected in from 50 to 70% of the trials due to random sampling. The mottled condition appears to be dominant to the clear or non-mottled seed coat and the results indicate that it is dependent, for its expression, on a single factor difference.

The 63 segregating F_3 progenies were tested for agreement with expectation, on the basis of a 3:1 segregation, both individually and collectively, by means of the X^2 distribution; X^2 for the .05 point and one degree of freedom is 3.841. Only 4 of the 63 progenies have values of X^2 greater than 3.841. The total X^2 for the 63 progenies is 61.2452. Since the X^2 table has not been calculated beyond N = 30, the goodness of fit was obtained by substituting in the formula $(\sqrt{2X^2} - \sqrt{2N-1})$. The X^2 test for large values of N is comparable to the t test in that the above formula gives a value which has a standard error of 1*; hence we may use the value obtained to enter the t table and obtain the probability in the usual way. The value obtained here is 0.11. This corresponds to a P value slightly greater than .9 which means that deviations as great or greater than the observed could be expected more than 90 times in 100 trials on the basis of random sampling.

A further test for goodness of fit was made, in which the observed distribution of the X^2 values of the 63 F_3 progenies was compared with the expected distribution. From these data (Table 1) a X^2 of 14.2222 was obtained. The corresponding P value for 6 degrees of freedom is between .05 and .02; hence in only 2 to 5 times in 100 trials would deviations as greater or greater than the observed occur, due to random sampling.

Examination of the data presented in Table 1 shows that six families fall within the range where P = 1.0. Furthermore the big contribution of X^2 is due to one group of twelve progenies falling within the range

TABLE 1.—TEST OF AGREEMENT OF THE OBSERVED DISTRIBUTION OF X^2 VALUES WITH EXPECTED FREQUENCIES IN 63 SEGREGATING F_3 PROGENIES FROM THE ALPHA NO. 5 X ARCTIC CROSS

X^2	P	C	O	O–C	$(O-C)^2$	$\frac{(O-C)^2}{C}$
.0000	1.00	.63	6			
.0002	.99	.63	0			
.0006	.98	1.89	0 6	−0.30	0.0900	0.014286
.0039	.95	3.15	0			
.0158	.90	6.30	8	1.70	2.8900	0.458730
.0642	.80	6.30	12	5.70	32.4900	5.157143
.148	.70	12.60	7	−5.60	31.3600	2.488889
.455	.50	12.60	8	−4.60	21.1600	1.679365
1.074	.30	6.30	11	4.70	22.0900	3.506349
1.642	.20	6.30	4	−2.30	5.2900	0.839683
2.706	.10	3.15	2			
3.841	.05	1.89	2			
5.412	.02	.63	2 7	0.70	0.4900	0.07778
6.635	.01	.63	1			

X^2 = 14.222223

* Statistical Methods for Research Workers—5th Ed. Pg. 62 by R. A. Fisher.

where $P = .80$. Hence the deviation from expectation is fairly significant and suggests that some factor or factors, which have not been taken into account, have influenced the classification of the F_3 progenies in such a way as to make the fit closer to a 3:1 than would be expected on the basis of random sampling.

Plant S-26-3-5, which was subsequently shown to be of hybrid origin, produced white flowers and heavily mottled seeds. Self-fertilized seeds were obtained from this plant in the greenhouse. A total of 148 F_2 plants were grown in two groups. The first group consisted of 61 plants which were sown in greenhouse flats and transplanted to the field nursery in the usual manner. Classification of the seeds from these 61 F_2 plants showed that 32 plants produced mottled seed and 29 produced clear seed. Self-fertilized seeds were obtained from 38 of the 61 F_2 plants.

F_3 progenies were grown from the 38 plants. Examination of the seeds from the plants in each progeny revealed that a number of F_2 plants, which showed not the faintest trace of seed coat pigmentation, produced F_3 progenies which segregated for mottled and clear seeded individuals. The F_2 ratio, corrected on the basis of breeding behaviour in the F_3, now gave 38 mottled to 23 clear seeded individuals.

This fact, together with the wide variation in segregation, shown by the different F_3 progenies, suggested that it would be advisable to examine seeds from a larger number of F_2 plants. This was possible since there was a quantity of reserve seed available from the original F_1 plant.

It was felt that the manner in which the F_2 plants were protected from cross-pollination in the field precluded any possibility of natural crossing. Nevertheless, in order to make certain that the peculiar F_3 ratios obtained were not the result of natural crossing, it was decided that additional F_2 plants should be grown to maturity in the greenhouse. Hence an additional F_2 progeny of 87 plants was planted and these matured seed during the winter months.

Examination of the seeds from the 87 F_2 plants showed that 47 plants produced mottled seed and 40 produced clear seed. This ratio was later corrected on the basis of breeding behaviour in the F_3 and finally gave 63 mottled to 24 clear-seeded individuals. Sixteen F_2 plants, which were originally classified as having clear seeds, produced F_3 progenies which consisted of clear seeded and of mottled seeded individuals.

The ratio resulting from totalling the two F_2 progenies was 101 mottled to 47 clear. Only two F_3 progenies consisted wholly of mottled-seeded plants. This segregation was tested for goodness of fit to a 3:1 ratio by means of the X^2 distribution and a X^2 of 1.1712 was obtained. This corresponds, for one degree of freedom, to a P value of between .2 and .3. Hence agreement of observed with expectation is satisfactory since deviations as great as the observed could be expected in 20 to 30% of the trials on the basis of random sampling. This corresponds with the ratio obtained in the S-30-58 material and indicates that in line S-26-3-5 also the mottled condition of the seed is at least partially dominant to the clear seed coat and that the mottled character is dependent upon a single factor difference.

F_3 progenies were grown from each of the 87 additional F_2 plants. Some of the F_3 progeny plants were grown in the field nursery and some in the

greenhouse. The greenhouse and field data have been combined for each progeny and the first group of F_3 progenies has been included also.

The wide variation in the proportion of plants which developed pigmented areas, as between the different F_3 progenies, is most striking. The second lots of progenies grown in the field nursery and in the greenhouse were equally as variable as the first group. In order to obtain additional data relating to the peculiar F_3 segregations, F_4 progenies were grown from several plants out of each of four F_3 progenies, from which selfed seeds were obtained in the greenhouse. In Table 2 the segregations obtained in the F_4 progenies are shown as well as the classifications of the F_3 parent

TABLE 2.—A COMPARISON OF THE CLASSIFICATION OF PLANTS FROM FOUR F_3 PROGENIES ON THE BASIS OF SEED EXAMINATION, WITH THE CLASSIFICATION BASED ON BREEDING BEHAVIOUR IN THE F_4

Classification of F_3 Parent Plant	Classification of F_4 Progeny M	C	Classification of F_3 Parent Plant	Classification F_4 Progeny M	C
F_3 = 45M:15C			F_3 = 15M:15C		
M	23	7	C	13	10
M	12	17	M	5	4
M	1	22	M	21	7
C	0	18	C	3	20
M	21	9	C	14	13
M	24	2	C	0	19
M	26	0	C	1	28
C	3	26	M	18	7
M	16	9	C	0	17
C	11	8	M	3	26
C	0	21	M	20	2
C	0	27	M	16	12
M	12	17	C	9	20
M	21	8	M	20	8
C	0	24	M	19	3
M	12	7	M	12	15
M	15	8	M	19	8
M	20	3	M	4	7
F_3 = 19M:13C			F_3 = 3M:20C		
C	0	18	C	0	26
M	11	12	C	5	24
M	20	4	C	13	17
C	6	15	C	0	19
M	3	6	C	0	26
C	2	22	M	8	21
C	16	7	C	14	9
M	10	6	C	0	17
M	19	0	C	0	29
M	24	0	M	3	22
M	16	5	C	19	7
M	12	9	C	0	25
M	16	8	C	22	3
C	0	24	C	0	16
M	5	18	C	0	21
C	3	19	C	0	10
M	8	8	C	19	6
C	1	22	C	0	25
C	11	13	C	0	21
M	16	7	C	3	13

NOTE:—M = Mottled
C = Non-mottled or clear.

plants from which the progenies were grown in each case. These segregations not only show striking variations, as between different plant progenies, but here again several F_3 plants, whose seeds showed no trace of pigmentation, produced F_4 progenies consisting of mottled and of clear-seeded plants.

Classification of Material

Difficulties were encountered in classifying the mottled seeded material on the basis of examination of the seed coats. All degrees of mottling were found from almost completely pigmented seed coats to the presence of only one or two tiny specks. Not infrequently all degrees were present in the seeds from a single plant. Other plants produced seeds of which 99% or more were clear and 1% or less showed only the faintest trace of pigmentation. In still others all of the seeds were distinctly pigmented.

Wherever a single pigmentated seed was found the plant was classified as mottled. During the earlier part of the study the mottling was graded as heavy, medium and light. As the study progressed, however, it became evident that there was nothing to be gained by such grading, since tests showed that there was no tendency for heavily mottled seeds to produce plants having heavily mottled seeds, nor for lightly mottled seeds to produce plants having lightly mottled seeds.

In line S-30-58 classification was further complicated by the presence of brownish discolored spots on some of the seeds. In some cases it was difficult to distinguish these from spots of pigment.

The Influence of Environmental Factors on Mottling

Tests were conducted to determine the influence of light, moisture, temperature, soil fertility and position of seeds on the plant upon mottling.

The use of heavy brown paper bags in obtaining self-fertilized seeds from some plants provided an opportunity to compare the open-pollinated seeds, which were matured in the light, with the selfed seeds which were matured inside the bags. In no case was there any noticeable difference in the percentage of the seeds which were pigmented or in the degree of pigmentation of the individual seeds, due to the effect of light.

To study the effect of moisture, cuttings were made from each of five plants. These were rooted in sand and later transplanted to large pots. Comparable cuttings were grown with an abundance of moisture and with little moisture. Comparison of seeds from the two treatments revealed no marked effects on the development of pigment in the seed coats. Similar cuttings were grown in temperatures ranging from 57° F.—63° F. in one case and from 70° F.—86° F. in another. No marked differences in seed mottling were observed between these two lots of plants.

To determine the effect of soil fertility a progeny of plants, known to be homozygous for the mottled condition, was grown. Two distinct soils, the analyses of which are shown below, were used.

Soil sample no.	% CaO	% Mg. O	% K_2O	P_2O 5%	N %	Organic matter %
I	1.82	0.97	0.70	0.17	0.68	11.60
II	0.45	0.54	0.60	0.04	0.09	1.20

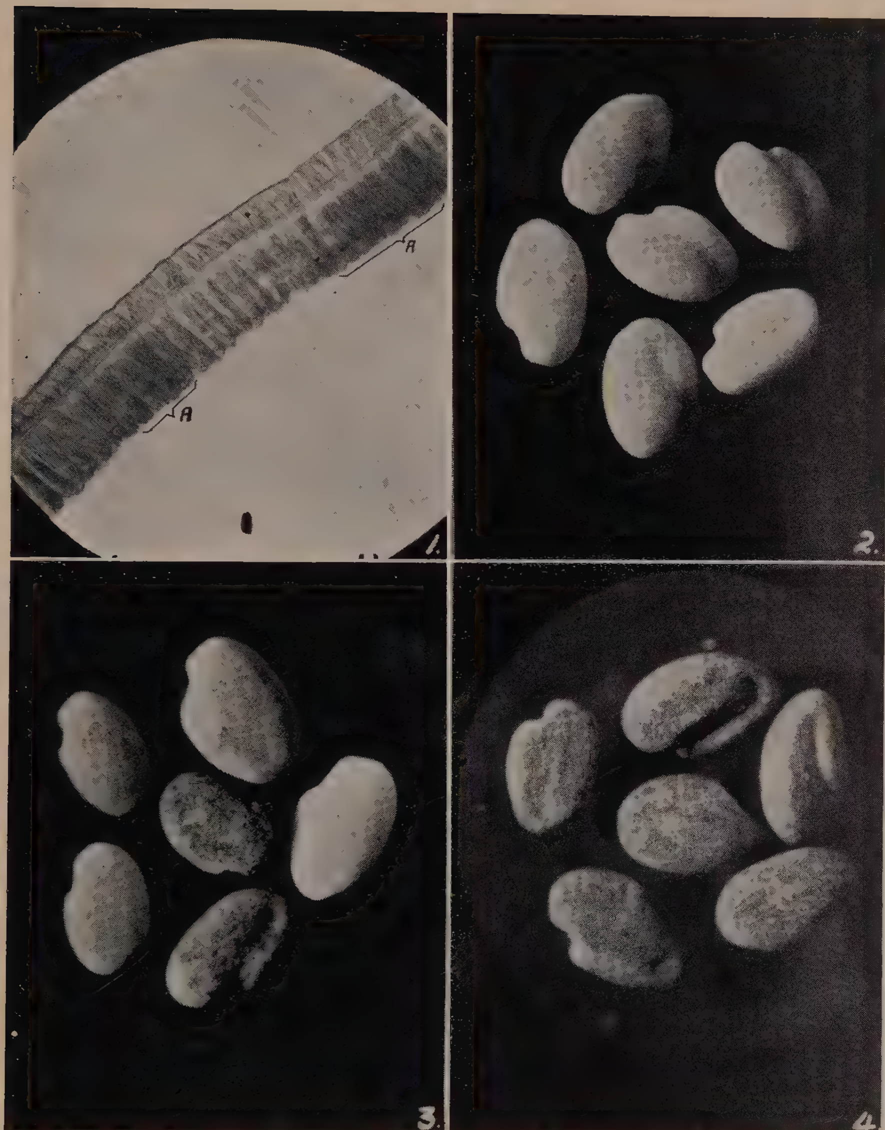

Figure 1. Section of seed coat from mottled seed. At "A" is shown location of pigment in lower parts of the Malphigian cells x 108.

FIGURE 2. Normal non-mottled seeds of *M. alba* x 11.

FIGURE 3. Seeds from a single plant of *M. alba* showing various degrees of mottling x 11.

FIGURE 4. Heavily mottled seeds of *M. alba* from line S-26-3-5. x 11.

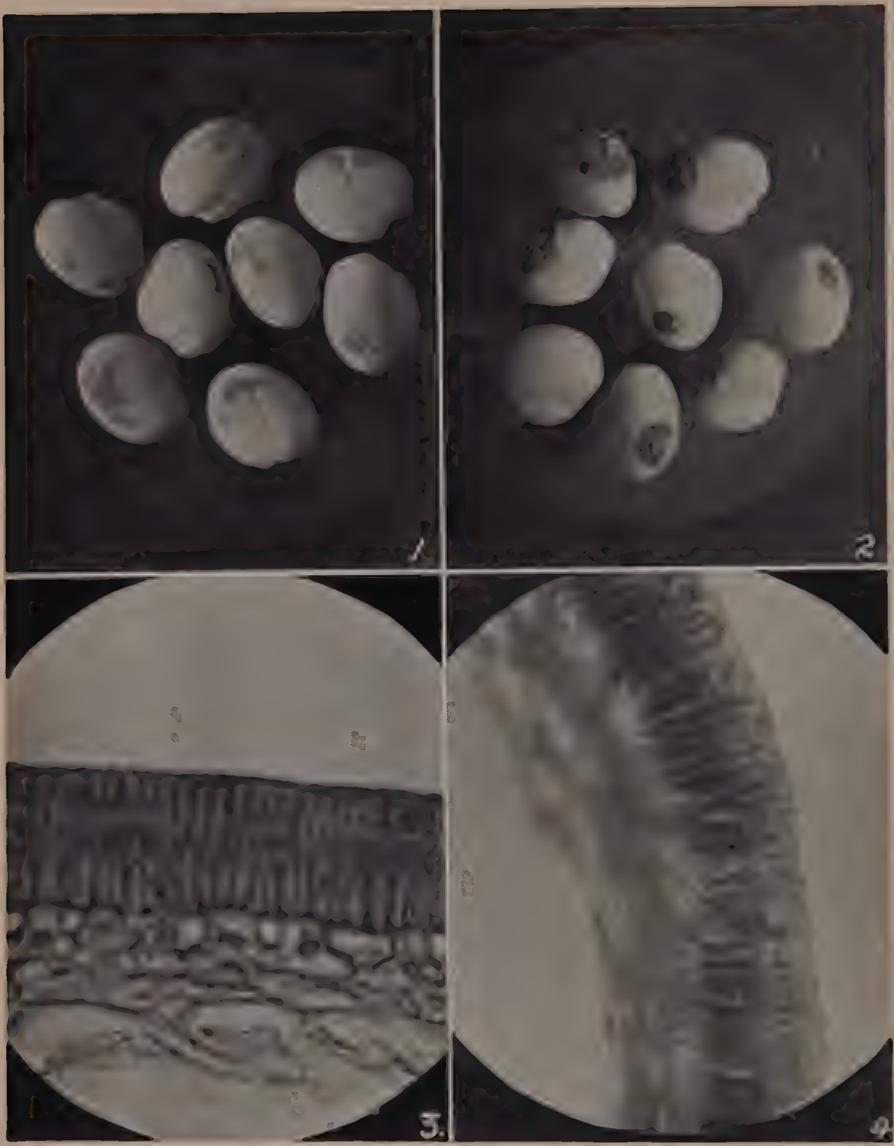

FIGURE 1. Permeable seeds showing discoloured areas. x 11.

FIGURE 2. Naturally permeable seeds shown in Fig. 1. after treatment in a one percent solution of osmic acid. x 11.

FIGURE 3. Section of the seed coat from an impermeable seed. x 108.

FIGURE 4. Section of seed coat through a discoloured area, as shown in Figure 1. x 108.

Forty plants were grown in each of the above soils. In addition, ten plants were grown on No. 2 soil, to which a heavy application of well rotted barnyard manure was added, and a similar number on the same soil to which ammonium phosphate, 150 lbs. per acre, was added. An examination of the seeds from the plants on these various tests failed to reveal any noticeable differences in the development of pigment.

An examination of seeds harvested from various positions on the racemes and from various racemes on a single stem failed to reveal any constant tendency in relation to pigment development. A nlmber of plants produced some pods which contained two seeds each; 208 of such pods were examined. In some cases both seeds were mottled; in other cases both were clear and in still other cases one was mottled and one clear.

The Location and Nature of the Pigment in Mottled Seeds

A study of heavily mottled seeds which had been immersed in water for several hours showed that only in the soft coated seeds, which had absorbed water and swollen, did the pigment disappear. Furthermore, when the seed coats of the hard seeds were pricked and replaced in the water they began to enlarge within a few hours and the pigment gradually disappeared from the seed coats. The results of these preliminary tests suggested that the pigment was not located in the cuticle nor in the matrix, which are readily permeable to moisture, but in some part of the seed coat located beneath the naturally impermeable region.

In order to determine the exact location of the pigment, sections of the seed coats were removed from dry untreated seeds and were embedded directly in paraffin and sectioned at a thickness of 15 μ. The paraffin was then removed with Xylol and the sections were mounted and examined at once. The brownish red pigment was found to be located in the lower portions of the Malphigian cells. No pigment was present in the "Cones" or those portions of the cells above the light line. A photomicrograph of a section of the seed coat showing the location of the pigment is shown in Plate I, Figure 1.

In order to determine the nature of the pigment concerned, a number of heavily mottled seeds were pricked and placed in a small amount of distilled water. After the mottling had disappeared the water was drained off and tested with glacial acetic acid. It gave the characteristic pink colour indicative of anthocyanin. A few drops of acetic acid added to the soaked seeds caused them to turn pink almost immediately. Some non-mottled seeds were treated in the same manner but gave a negative reaction when treated with glacial acetic acid. Clear seeds from plants which also produced some mottled seeds were also tested. They gave no indication of pinkish colour after the glacial acetic acid was added.

It was thought that this test might be of value in classifying some of the material included in the inheritance study of the mottled character. Reserve seeds of several of the F_2 plants, Line S-26-3-5, which were classified originally as having clear seeds but which produced F_3 progenies that segregated for mottling, were tested. The results were negative. If colour was developed it was so slight as to be of no value in classifying the material.

Inheritance of the Dwarf Branching Character

Since the material that was used in the study of the mottled seed coat of sweet clover, lines S-30-58 and S-26-35, segregated also for dwarf branching and common sweet clover types of plants, an opportunity was afforded to study the mode of inheritance of the dwarf branching character.

F_1 plant No. S-30-58 was of the type common to *M. alpha*. It possessed a few stems which were quite coarse and tall. Since the female parent of this plant was of the dwarf branching type, it is evident that the common type of plant is dominant to the dwarf branching type in this cross.

The F_2 progeny, consisting of 148 plants, was classified on the basis of plant type: 104 plants were classified as common type, and 44 as dwarf branching type. There were 4 plants which were recorded as dwarf branching type that were regarded as doubtful. They possessed more stems than is usual for the common type plants, but fewer than is common in the dwarf branching type. Classification of the F_3 progenies from these plants showed that they should have been recorded as common type. Hence on the basis of breeding behaviour in the F_3, the F_2 ratio was corrected to 108 common type and 40 dwarf branching type. This ratio was tested for goodness of fit on the basis of a 3:1 segregation, by means of the X^2 distribution and a X^2 of .3243 was obtained. For one degree of freedom this corresponds to a P value of between .50 and .70; hence a worse fit might be expected on the basis of chance 5 to 7 times in ten trials. The agreement of the observed to the expected on the basis of a 3:1 ratio is in this case satisfactory.

Self-fertilized seeds were obtained from 108 of the F_2 plants and F_3 progenies were grown from these. The dwarf branching type of F_2 plants produced F_3 progenies which, without exception, consisted wholly of dwarf branching type plants. The common type plants produced progenies of two types: those which were composed wholly of common type plants, and those which segregated for both types of plants.

The agreement with expectation of 54 F_3 progenies on the basis of a 3:1 ratio was tested individually and collectively by means of the X^2 distribution. Only a small percentage of the progenies gave X^2 values in excess of 3.841, which corresponds to the .05 point and one degree of freedom in Fisher's tables. By substituting in the formula $(2\sqrt{2 \times 2} - \sqrt{2N-1})$ a value of .5484 was obtained. Referring to Fisher's table of t the corresponding P value is approximately .6, which indicates that the agreement is very good.

Table 3 shows the observed distribution of X^2 for the 54 segregating F_3 progenies compared with the expected distribution. Here X^2 has a value of 9.333518 and the corresponding P value for 6 degrees of freedom is between .20 and .10. Hence the deviation from expectation would be exceeded by chance in 10 to 20% of the cases, due to random sampling. The common type of growth appears to be completely dominant to the dwarf branching type and the latter is probably dependent for expression on a single factor difference.

It has already been stated that plant S-26-3-5 was of the common sweet clover type. F_2 progeny plants, totalling in all 148, were grown; 115 of these were of the dwarf branching type, and 33 were of the common type. This ratio was tested for goodness of fit to a 3:1 segregation, by the X^2 distribution. An X^2 of .5765 was obtained for 1 degree of freedom, which

TABLE 3.—TEST OF AGREEMENT OF THE OBSERVED DISTRIBUTION OF X^2 VALUES WITH EXPECTED FREQUENCIES IN 54 SEGREGATING F_3 PROGENIES

X^2	P	C	O	O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
.0000	1.00	.54	1			
.0002	.99	.54	0 } 1	−4.40	19.3600	3.585185
.0006	.98	1.62	0			
.0039	.95	2.70	0			
.0158	.90	5.40	7	−1.60	2.5600	.474074
.0642	.80	5.40	8	−2.60	6.7600	1.251852
.148	.70	10.80	6	−4.80	23.0400	2.133333
.455	.50	10.80	12	1.20	1.4400	.133333
1.074	.30	5.40	7	1.60	2.5600	.474074
1.642	.20	5.40	5	−0.40	.1600	.029630
2.706	.10	2.70	4			
3.841	.05	1.62	2 } 8	2.60	6.7600	1.251852
5.412	.02	.54	1			
6.635	.01	.54	1			

$X^2 = 9.333518$

corresponds to a P value of between .30 and .50. Hence a deviation as great as the one obtained might be expected to occur due to chance 3 to 5 times in 10 trials.

Selfed seeds were obtained from 125 of the F_2 plants and F_3 progenies were grown from these. All of the progenies grown from dwarf branching type plants consisted wholly of dwarf branching type individuals. F_3 progenies from common type plants were of two types: 74 of these segregated for both dwarf branching and common types of plant, and 29 consisted of common type plants only.

The agreement with expectation of 74 F_2 progenies on the basis of a 3:1 ratio was tested by means of the X^2 distribution.

Total X^2 for $N = 74$ was 71.45. Substituting in formulae $(\sqrt{2X^2} - \sqrt{2N-1})$ we get a value 0.1685. Referring to Fisher's t tables we find that this corresponds to a P value of between .8 and .9, which indicates that agreement with expectation is very good.

In Table 4 the observed distribution of X^2 values is compared with the expected on the basis of a 3:1 segregation for plant type. For $N = 6$ a

TABLE 4.—TEST OF AGREEMENT BETWEEN OBSERVED AND EXPECTED DISTRIBUTIONS OF X^2 VALUES APPLIED TO 74 SEGREGATING F_3 PROGENIES (LINE S-26-3-5) ON THE BASIS OF A 3:1 SEGREGATION FOR COMMON AND DWARF BRANCHING TYPES OF PLANTS

X^2	P	C	O	O–C	$(O–C)^2$	$\frac{(O–C)^2}{C}$
.0000	1.00	.74	2			
.0002	.99	.74	0 } 4	3.40	11.5600	1.562163
.0006	.98	2.22	0			
.0039	.95	3.70	2			
.0158	.90	7.40	9	1.60	2.5600	.345946
.0642	.80	7.40	10	2.60	6.7600	.913514
.148	.70	14.80	16	1.20	1.4400	.097297
.455	.50	14.80	15	.20	.0400	.002703
1.074	.30	7.40	5	2.40	5.7600	.778378
1.642	.20	7.40	4	3.40	11.5600	1.562166
2.706	.10	3.70	5			
3.841	.05	2.22	4 } 11	3.60	12.9600	1.751354
5.412	.02	.74	0			
6.635	.01	.74	2			

$X^2 = 7.013521$

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X^2 value of 7.013521 was obtained. By referring to Fisher's tables of X^2 we find the corresponding P value for 6 degrees of freedom to be between .30 and .50; hence in from 3 to 5 trials out of every 10 we might expect worse agreement than that obtained due to chance alone. Hence the data obtained from this material indicates that the dwarf branching character is recessive and depends for its expression on a single factor difference.

Tests for Independence

The F_2 plants from lines S-50-38 and S-26-35 were classified with respect to seed coat markings and with respect to plant type. The data obtained were tested for independence with respect to the above characters. The X^2 test for independence in line S-30-58 gave a X^2 of 0.1195 and in line S-26-35 a X^2 of 1.1065. In both cases independence is clearly indicated.

Hard Seed Study

Seeds from each of the single plant selections made in the fall of 1931 were subjected to germination tests, in order to determine the proportion of naturally permeable seeds in each selection. Results from tests made on a total of 10,982 selections are shown in Table 5.

TABLE 5.—FREQUENCY DISTRIBUTION OF SELECTED PLANTS ON THE BASIS OF THE PERCENTAGE OF NATURALLY PERMEABLE SEEDS PRODUCED

Percentage of permeable seeds	0	1	2	3	4	5	6	7	8	Total
No. of plants..........	7,293	1,632	1,428	51	442	68	17	0	51	10,982
Percentage of plants.....	66.40	14.86	13.00	0.47	4.02	0.62	0.16	0	0.47	—

These data show that 66.4% of the plants included in this test produced only hard or impermeable seeds which would normally require scarification or other treatment to render them permeable before they would germinate. Furthermore 94.26% of the selections fell within the range of 0 to 3% permeable seeds and the average percentage of permeable seeds in the 10,982 selections tested was only 0.66%.

Since these selections were taken at random from a number of different fields, these data undoubtedly provide a fairly accurate estimate of the extent to which naturally hard seeds occurred in common white blossom sweet clover, *M. alba*, under the conditions existing in 1931 in the Saskatoon area.

A second lot of selections, consisting of 360 individual plants, were made from single plant progenies out of the Arctic variety. The plants, from which these progenies were grown, were selected from bulk arctic in 1929 and were open-pollinated. The selections made in 1931 were chosen at random from open-pollinated plants within each progeny. The percentage of permeable seed from each selection was determined and the data are shown in Table 6.

These 360 selections produced an average of 3.49% permeable seeds. An examination of the data in Table 6 shows that a few of the selections

TABLE 6.—FREQUENCY DISTRIBUTION OF 360 SELECTIONS OUT OF SINGLE PLANT PROGENIES OF ARCTIC SWEET CLOVER ON THE BASIS OF PERCENTAGE OF PERMEABLE SEEDS PRODUCED

Percentage of permeable seed Progeny No.	Number of plants											Total
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	
0	11	1	14	10	18	23	2	30	4	29	19	161
1	26	—	3	4	—	—	—	—	—	1	—	34
2	10	1	3	12	9	3	6	3	1	—	9	57
3	7	1	3	—	—	—	10	—	—	—	—	21
4	6	2	3	—	3	1	13	—	1	1	3	33
5	1	5	2	—	—	—	6	—	1	—	—	15
6	—	6	2	—	—	—	—	—	—	—	—	8
7	—	4	—	—	—	—	2	—	—	—	—	6
8	—	—	—	—	—	—	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—	—	—	—	—
10–14	—	—	1	—	2	—	2	—	1	—	—	6
15–19	—	1	—	—	1	—	1	—	—	1	—	4
20–29	—	—	1	—	—	—	—	1	1	1	1	5
30–39	1	1	—	—	1	—	—	—	1	—	—	4
40–49	—	—	—	—	—	—	—	—	1	—	—	1
50–59	1	—	1	—	—	—	—	—	—	—	—	2
60–69	—	—	1	—	—	—	—	—	—	—	—	1
70–79	—	—	—	—	—	1	—	—	—	—	—	1
80–89	—	—	—	—	—	—	—	—	—	—	—	—
90–100	1	—	—	—	—	—	—	—	—	—	—	1
Total..........												360

produced unusually high proportions of seeds which absorbed water and germinated readily.

The seeds from a number of the highly permeable selections were examined carefully under high power binoculars to determine whether the permeable condition was associated with observable seed coat peculiarities. The seed coats appeared normal in all cases and the seeds appeared sound, well matured and bright in colour.

Progenies were grown from all of the original selections which produced ten per cent or more permeable seeds, as well as from a few selections which produced only hard seeds. The progeny plants were started in greenhouse flats and were later transplanted to the field nursery. These plants were all bagged or caged and self-fertilized seeds were obtained. Seeds from open-pollinated flowers were also harvested and were tested for seed coat permeability after the manner described for the original selections.

On the basis of the data obtained from these tests, selections were made from the first generation inbred plants. Out of the progenies arising from highly permeable original selections, only the most highly permeable individuals were chosen and out of the progenies from original selections, which produced only hard seeds, only individuals which did not produce any permeable seeds were selected from which to grow second generation inbred progenies. The testing of seeds from progeny plants and selection on the basis of the percentage of permeable seeds was thus carried on continuously within inbred lines.

Several progenies grown from first generation inbred plants failed to produce seeds. The leaves of these plants appeared thick and leathery and were curled at the edges. Flowers were produced irregularly and never opened normally. These progenies all originated from selections taken out of one line. It was necessary to discontinue selection within those progenies. In the progenies from the five remaining lines there were no plants of abnormal appearance. Flowering and seed setting appeared normal and the plants were strong and vigorous in appearance.

The effect of continuous selection within the inbred lines of the most highly permeable individuals and of the individuals producing non-permeable seeds, for two successive generations, is shown in Table 7.

In sections B and C of Table 7 the data for each line represent an average of all progenies grown from selections out of that line.

Figures 1 and 2 show graphically the effects of continuous selection of individuals producing high proportions and low proportions of permeable seeds respectively. The data for all of the five lines were averaged in preparation of the graphs.

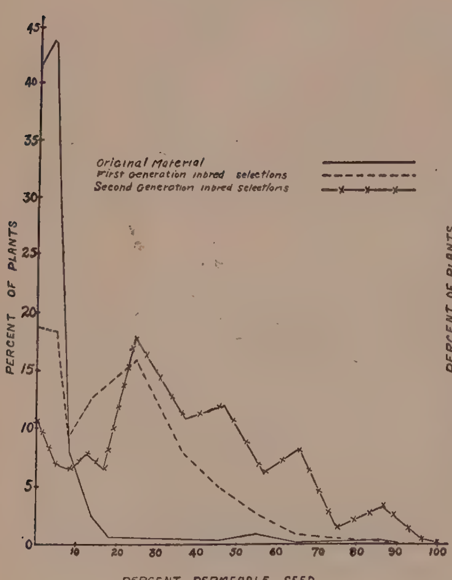

FIGURE 1. The effect on seed coat permeability of two successive generations of selection of individuals producing high percentages of permeable seeds.

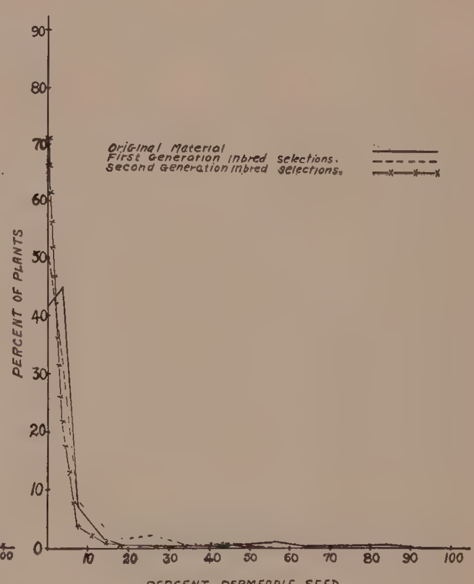

FIGURE 2. The effect on seed coat permeability of two successive generations of selection of individuals producing only hard seeds.

These data show a progressive increase in the proportion of individuals which produce large percentages of permeable seeds in the generations obtained successively from individuals producing highly permeable seed and a progressive diminution in the percentage of individuals which produce permeable seeds in the generations obtained successively from individuals, which produce only hard seeds.

TABLE 7.—THE EFFECT OF SELECTION FOR TWO SUCCESSIVE GENERATIONS, UPON THE PERCENTAGE OF PLANTS PRODUCING DIFFERENT PROPORTIONS OF PERMEABLE SEEDS

SECTION A—Lines from which original selections were made

No. of line from which original selections were made	Proportion of permeable seeds in per cent													No. of plants tested	Average percent of permeable seeds
	0	1-4	5-9	10-14	15-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-100		
I	17.19	76.37	1.56	—	—	—	—	1.56	—	—	—	1.56	—	64	4.26
II	21.05	42.11	26.32	—	5.26	—	5.26	—	—	—	—	—	—	19	5.36
III	55.56	22.22	7.41	3.70	—	3.70	—	—	7.41	—	—	—	—	27	5.85
IV	38.47	61.53	—	—	—	—	—	—	—	—	—	—	—	26	0.82
V	48.39	41.94	3.23	6.45	—	—	—	—	—	—	—	—	—	31	2.00

SECTION B—Two successive generations of selection of individuals producing high percentages of permeable seeds

I	39.47	7.90	5.26	5.26	5.26	18.42	2.63	2.63	5.26	7.90	—	—	—	38	16.47
II	15.13	22.69	23.53	7.56	2.52	6.72	4.20	—	5.88	5.04	—	5.88	0.84	119	19.16
III	6.34	2.61	2.24	7.84	7.46	22.39	13.06	16.79	8.21	8.96	1.49	2.61	—	268	33.36
IV	6.25	5.00	2.92	9.58	7.08	16.67	14.17	15.00	5.42	9.58	1.67	6.25	0.42	240	28.17
V	19.27	3.61	8.43	4.82	9.64	20.48	12.05	14.46	4.82	2.41	—	—	—	83	22.05

SECTION C—Two successive generations of selection of individuals producing only hard seeds

I	80.00	10.91	1.82	5.45	1.82	—	—	—	—	—	—	—	—	55	0.90
II	83.92	15.18	0.89	—	—	—	—	—	—	—	—	—	—	112	0.53
III	69.35	15.32	5.65	1.61	1.61	4.03	1.61	0.81	—	—	—	—	—	124	3.12
IV	79.49	20.51	—	—	—	—	—	—	—	—	—	—	—	39	0.61
V	64.71	26.47	8.82	—	—	—	—	—	—	—	—	—	—	34	1.23

Cause and Nature of Seed Coat Permeability

Hamly (7) examined naturally permeable seeds of *M. alba* and found that the area of absorption of moisture was invariably located at the strophiole. In no case did he find that any particular region of the seed coat, other than the strophiole, accounted for the naturally permeable condition.

Seeds from 16 highly permeable selections out of lines, having high average permeability, and from 12 highly impermeable selections out of lines, showing extremely low average permeability, were studied and comparisons were made with reference to the structure of the seed coats and location of naturally permeable areas. Seeds from each of the 16 highly permeable selections were treated with a 1% solution of Osmic acid, as described by Hamly (7), to determine the location of permeable areas. Of the 16 selections tested, 15 showed the point of penetration of the acid to be at some part of the seed othe: than the strophiole. In one line only there were a few seeds which were permeable at the strophiole. This test showed that with the possible exception of one line permeability, in this material, was generalized in the whole seed coat and was not due to a change in any particular region. Seeds from the impermeable selections were also treated with Osmic acid, but showed no indications of permeability in any part of the seeds.

Random samples of seed from six of the highly permeable selections were treated with sulphuric acid (Sp. gr. 1.84) for the intervals of time stated. After treatment the acid was washed off and the seeds were soaked in water for four hours. The proportions of permeable and non-permeable seeds were then ascertained. The results are summarized in Table 8.

These data show clearly that the "hard" seeds in the permeable lines are quite different from the "hard" seeds from the impermeable lines. In the case of lines I and II a treatment of five minutes duration produced a very pronounced increase in the proportion of permeable seeds and the twenty minute treatment caused almost all of the seeds to become permeable in each of the five lines.

The results from five non-permeable lines showed that five minutes treatment caused only a few seeds in one line to become permeable and none in the other four lines. Treatments of twenty minutes duration resulted in an average of only 15.6% permeable seeds.

It is quite evident that the hard seeds from the highly permeable lines have seed coats that are much more readily made permeable than those of seeds from the non-permeable lines. Hence it was thought probable that many of them would germinate under field conditions. In order to determine if this were the case a quantity of seeds from each of the six permeable plants, listed in Table 12, were placed in water at room temperature. They remained in the water for ten hours and at the end of that time all of the seeds which had absorbed moisture and were swollen were removed. The remaining pseudo-hard seeds were then taken and planted in moist soil in the greenhouse. At the end of twenty-one days 86% of the seeds had germinated and the seedlings had emerged. Hence testing these seeds by soaking them in water does not provide a reliable estimate of their

TABLE 8.—SULPHURIC ACID TEST OF SWEET CLOVER SEED

Strain No.	Percentage of permeable seeds as shown by original germination test	Time in H_2SO_4 minutes	No. of seeds permeable after H_2SO_4 treatment	No. of seeds non-permeable after H_2SO_4 treatment	Percentage of seeds permeable after H_2SO_4 treatment
I	54	5	70	7	90.9
		15	51	1	98.1
		22	87	0	100.0
II	62	5	54	8	87.1
		15	51	0	100.0
		22	70	0	100.0
III	36	5	13	57	18.6
		10	30	39	43.5
		15	62	7	89.8
IV	56	5	31	18	63.2
		10	29	20	39.2
		15	38	13	74.5
		25	48	2	96.0
V	36	5	19	51	27.1
		12	51	19	72.9
		20	68	2	97.1
VI	48	5	23	48	32.3
		12	57	12	82.6
		20	68	1	98.5
Ia	0	5	0	50	0.0
		15	2	48	4.0
		22	22	76	22.4
IIa	0	5	0	70	0.0
		10	2	72	2.7
		15	2	63	3.1
IIIa	0	5	2	68	2.7
		12	3	68	4.2
		20	13	61	17.5
IVa	0	5	0	70	0.0
		12	5	65	7.1
		20	11	59	15.7
Va	0	5	0	70	0
		12	1	69	1.4
		20	5	65	7.1

permeability under field conditions. Hard seeds from highly impermeable lines showed no tendency to germinate when treated in a similar manner.

A histological examination of the pseudo-hard seeds from permeable lines and of the hard seeds from impermeable lines, which were treated with sulphuric acid, was made. In both cases the acid dissolved away the subcuticular layer to the "light line." Hence the "light line" appears to be the real region of impermeability. No dye was found that would stain the light line and it was not possible to observe any differences in structure in that region, as between pseudo-hard and hard seeds.

Sections through the naturally permeable areas of seeds from the highly permeable lines, treated with osmic acid, showed a marked difference in compactness of the cells, as compared with the impermeable areas. The osmic acid in the permeable seeds penetrated by way of the middle lamella.

In the second inbred generation progenies, from highly permeable selections out of permeable lines, a number of plants produced seeds which,

upon examination, were shown to have brownish areas on the seed coats. Under high power binoculars these areas appeared to be distinctly sunken below the normal seed coat area and to have very irregular surfaces.

Treatment of these seeds with osmic acid shows that the discoloured areas coincide with the areas of absorption of moisture. Permeable seed coats, however, were not confined alone to those seeds which possessed discoloured areas. Many normal appearing seeds were shown to be permeable.

These seeds possessing the discoloured areas were examined histologically. In sectioning the seeds the discoloured areas appeared to lack cohesion. Very thin sections invariably disintegrated in the process of making the slides. Sections of 25 μ or thicker were therefore examined. A comparison of the sections from the normal and discoloured areas shows considerable difference in all layers of the seed coat. It is evident that the characteristics of the normal seed coat, which render it non-permeable, are lacking in these discoloured areas. The cuticle is frequently broken; the matrix is thinner than normal. The light line is very indistinct. The Malphigian cells are shorter and clefts are apparent between them and the osteosclerid cells are mostly collapsed. A comparison between the structure of the normal seed coat and a discoloured area is shown in Plate II, Figure 1.

The cause of these discoloured areas on the seeds has not been ascertained. In some of the sections examined bits of tissue resembling Mycelia were observed. Whether these actually were fungus mycelia could not be definitely determined but if the condition is due to fungous growth in the seed coat, it is evident that the fungus has in no way interfered with the growth, flowering or seed setting of the plants.

DISCUSSION OF RESULTS

Results from investigations dealing with the inheritance of the mottled condition of the seed coats of *M. alba*, in the cross Alpha X Arctic and in a natural hybrid which produced progenies that segregated for mottled and non-mottled seed coats, indicate that the mottled character is dependent upon a single factor pair. The non-mottled condition appears to be recessive and the development of pigment in the coats of seeds, which carry the factors necessary for mottling, appears to be influenced by other factors which have not been determined.

Classification of the materials involved in this study was difficult. Most of the plants, which produced mottled seeds, also produced clear seeds. Not infrequently less than 5% of the seeds from a single plant showed mottling, the remainder being clear. The degree of mottling varied greatly, within the seeds, from any one plant. In a number of cases F_3 progenies, which produced both mottled and clear seeded individuals, were produced from clear seeded F_2 plants; hence classification, based upon the presence or absence of pigment in the seed coats, does not always indicate the genotype with regard to the mottled character. All F_2 classifications made on the basis of appearance of the seed coats were changed somewhat when the breeding behaviour of the F_3 progenies was determined. Development of pigment in the seed coats of the F_2 plants, grown from line S-30-58, was much more regular than in those grown from line S-26-3-5.

Histological studies made on the seed coats of mottled seeds show that the pigment is located in the lower parts of the Malphigian cells. Since this portion of the seed coat is maternal tissue, being derived from the outer integument of the ovule, the cells supposedly all have the same hereditary potentialities. Hence, if the development of pigment depended solely on the hereditary constitution, one would expect all to be of the same colour.

The fact that there is no tendency for heavily mottled seeds to produce plants having heavily mottled seeds nor for lightly mottled seed to produce plants having seeds of like kind shows that the variation in the degree of mottling is not inherited. This fact is further indicated by the occurrence of plants with mottled seeds in inbred progenies, grown from F_2 plants, which produced only clear seeds. Hence, it is evident that the development of pigment in the seed coat is influenced greatly by other factors.

A study of the effect of various environmental factors, including light, temperature, moisture and soil fertility, failed to show that any of these factors markedly influenced the development of pigment in the seed coats of sweet clover. These results are similar to those obtained by Hollowell (8), Woodworth and Cole (24) and Owen (18) in their investigations relating to mottling in soybeans. However, these workers found that soil fertility had a marked effect on soybean mottling. No such effect was observed in the studies conducted with sweet clover.

The time of maturity of the seeds also appeared to have no effect upon the development of mottling in sweet clover seeds. Examination of seeds from different branches of a single plant, from different racemes on a single branch and from different locations on a single raceme all showed similar variations with regard to degree of mottling. A number of pods, bearing two seeds each, were examined. Of those produced by a single plant some contained one seed which was distinctly mottled and one which carried no trace of mottling. Others contained seeds which were both mottled or both non-mottled. Hence it is evident that the development of pigment in the seed coat has little relationship to the time of maturity of the seeds.

The fact that seeds of common biennial white blossom sweet clover may be potentially mottled, and still show no trace of pigmentation of the seed coat, is a matter of some importance in the production of registered and certified seed. Growers may purchase foundation stock seed showing no trace of mottling and find that the seed crop grown from it contains sufficient mottled seed to prevent it from being registered or certified, as the case may be. On the other hand, if the presence of mottled seeds in a sample of white blossom sweet clover seed is regarded as evidence of an admixture of yellow flowered species the seed grower may unjustly be compelled to suffer a loss.

Mottled seeds, when scarified, soaked and treated with glacial acetic acid, turned reddish or pinkish in colour. The pigment was shown to be readily soluble in water by the fact that mottled seeds, when scarified and placed in water, lost the mottled appearance. When glacial acetic aid was added to this solution in which the seeds were soaked, a decided pinkish coloration resulted. These pigments have all the general properties of anthocyanins.

The results of studies on the dwarf branching habit of growth, in a cross between Alpha 5 and Arctic and in a natural hybrid which produced progenies that segregated for dwarf branching and common type plants, indicate that it is dependent for its expression upon a single factor difference. The common type of growth is dominant to the dwarf branching type in the material studied.

Most of the recessive type plants could be identified in the early stages of growth by the marked tendency to branch from the crown nodes. A few plants, however, did not develop the typical branching habit until after they were transplanted to the field nursery. Hence the materials used in these studies were classified in the field nursery during late summer of the first year of growth and again prior to harvesting the seed in the second year. In only four of the mature plants was there any doubt as to the classification. These developed more stems than is usual for the common type but progenies grown from them showed that they were not of the dwarf branching type.

Since the materials used in this study segregated also for mottled and clear seeds, it was possible to determine the probability of the two being associated in inheritance. Analyses, by means of the X^2 test for independence, indicated that these characters were inherited independently.

Studies made on common white blossom biennial sweet clover in the Saskatoon area in 1931 showed that less than 1% of the seeds were naturally "soft" coated or able to absorb moisture and germinate without being scarified or otherwise treated to render them permeable. Several single plant progenies of the Arctic variety produced relatively high proportions of permeable seeds.

Investigations into the cause and nature of hard seeds in *M. alba* tend to confirm the findings of Coe and Martin (3) that the region of impermeability of the seed coat coincides with the "light line." The region, known as the light line in the seeds worked with, was well developed, as shown in Plate I, Figure 1, and in Plate II, Figure 3. The nature of this region is such that it does not stain readily. Treatment with concentrated sulphuric acid for periods of twenty minutes destroyed the cuticle, matrix and caps of the Malphigian cells, but had little effect upon the "light line." Most of the "hard" seeds were not rendered permeable by this treatment; hence it is evident that the "light line" is a well differentiated region and that it plays an important role in seed coat permeability.

Results obtained from selecting individuals, producing high proportions of permeable seeds for two successive generations, indicate that the soft coated or permeable condition of the seeds in *M. alba* is, in some cases at least, inherited and that strains, having permeable seeds, may be produced through continuous selection within inbred lines. In these studies selection was carried in both plus and minus directions and in each case striking increases were obtained in the direction in which selection was made. Similar results were obtained by Sengbush and Loschakowa (21) in their work with *Lupinus luteus*. They succeeded in isolating "soft coated" lines of this species. However, the results are at variance with those of Zenari (25) who found that selection for three successive generations did not influence the percent of hard seed in *Leguminosae*.

Treatment of the seeds from the permeable lines with a 1% solution of osmic acid shows that absorption of moisture takes place at various points on the seed coats and is not restricted to any particular region.

Histological studies revealed that the cells in the permeable areas are less closely packed than in the impermeable areas and that the penetration of moisture is by way of the middle lamella. There is a marked difference in the seed coats of hard seeds out of permeable lines and those out of impermeable lines. The former respond much more quickly to sulphuric acid treatment and many of them were found to germinate without treatment when sown in moist soil. Histological examinations failed to reveal any structural differences as between these pseudo-hard and hard seeds.

Second generation progeny plants from certain of the highly permeable lines produced some seeds which showed small brownish areas. Tests with osmic acid showed that these areas absorbed moisture readily. An examination of sections through these areas showed the cells to be somewhat disorganized and abnormal. Many of the seeds from these same plants showed no discoloured areas and were nevertheless permeable. The cause of the discoloured areas was not definitely determined. Seeds carrying these markings were not associated with any abnormal plant behaviour. The plants were vigorous in appearance and blossomed and set seed in the normal manner.

SUMMARY

1. Inheritance of the mottled condition of the seed coat in *M. alba* was studied in the F_2 and F_3 generations of crosses involving these characters.

2. The mottled condition was found to be inherited as a dominant character when other factors are favourable for the development of pigment and evidence is presented to show that a single factor pair is active in its determination.

3. Plants which are potentially mottled may or may not develop pigment in their seed coats. Seeds with clear and with pigmented coats are frequently found on the same plant.

4. Classification of the progeny plants in the mottled seed study could not be done accurately on the basis of appearance of the seed coats.

5. The pigment involved in mottling of these seeds has all of the general properties of anthocyanins.

6. Histological studies showed the pigment to be located in the lower parts of the Malphigian cells.

7. The inheritance of plant type in crosses between dwarf branching and common biennial white blossom sweet clover showed the dwarf branching character to be dependent upon a single factor difference.

8. The typical common sweet clover is dominant to the dwarf branching type of growth.

9. The majority of the dwarf branching type plants are readily recognized in the seedling stage, but a few individuals did not develop the typical branching from the crown until a later stage of growth was reached.

10. Analyses of data, from progenies which segregated for both mottled and clear seeds and for type of growth, showed that these characters were not associated in inheritance.

11. An examination of seeds from 10,982 plants of common white blossom sweet clover, grown in 1931, showed that 99.3% of the seeds were incapable of germination, due to impermeability of their seed coats.

12. Individual plant selections, which produced relatively high percentages of naturally permeable seeds, were obtained from the Arctic variety.

13. Selection within inbred lines of the plants which produced the highest proportions of naturally permeable seeds, for two successive generations, resulted in marked increases in the proportions of permeable seeds produced and in the proportion of plants producing permeable seeds.

14. Continuous selection of plants which produced only hard seeds resulted in a marked increase in the proportion of hard seeds in the second generation inbred lines.

15. Treatment of permeable seeds with osmic acid showed the permeable areas to be distributed at random and not confined to any particular region of the seed coat.

16. Histological examination of seeds treated with sulphuric acid showed the light line to be the real region of impermeability in hard seeds.

17. Apparently hard seeds selected from the highly permeable lines were rendered permeable by treatment with sulphuric acid, but only a small proportion of the hard seeds from impermeable lines responded to the same treatment.

18. Seeds from permeable plants, which did not germinate readily in laboratory tests, may be regarded as pseudo-hard. A high proportion of them germinated without treatment when sown in moist soil.

19. Seeds, having brownish spots, occurred in some of the plants within highly permeable lines. When treated with osmic acid, these discoloured areas were found to absorb the solution readily.

20. Histological examination of sections through the brownish areas showed that the cells of the seed coat were somewhat disorganized.

21. The naturally permeable seeds in those lines were not confined to only those which possessed the brownish areas. Many normal appearing seeds also absorbed water and germinated readily without being treated.

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Résumé

Étude du mélilot au point de vue du mode de végétation, de la pigmentation des semences et de la perméabilité de l'enveloppe de la semence. Trueman M. Stevenson, laboratoire de récoltes fourragères, Saskatoon, Sask.

La transmission héréditaire de l'état tacheté ou bigarré de l'enveloppe de la semence dans *M. alba* a été étudiée dans les générations F_2 et F_3 de croisements présentant ces caractères. On a constaté que cet état bigarré est transmis héréditairement comme caractère dominant lorsque les autres caractères sont favorables au développement du pigment, et des preuves sont présentées pour indiquer qu'un couple unique de facteurs est actif dans la détermination de ce caractère. Les plantes potentiellement tachetées peuvent développer ou ne pas développer de pigment dans la tunique de leur semence. On trouve souvent sur la même plante des semences à tunique claire et pigmentée. La classification des plantes de progéniture par l'étude de la semence bigarrée ne peut être faite exactement sur la base de l'apparence des enveloppes de la semence. Le pigment qui occasionne la bigarrure de ces semences a toutes les propriétés générales des anthocyanines. Une étude histologique a démontré que le pigment se trouve dans les parties inférieures des cellules Malphigiennes. La transmission héréditaire du type de la plante dans les croisements entre le mélilot nain branchu et le bisannuel commun à fleurs blanches a fait voir que le caractère nain et branchu dépend de la différence d'un seul facteur. Le mélilot commun typique est dominant dans le type à végétation naine, branchue. La majorité des plants à type nain et branchu se reconnaissent facilement dans la phase de la

plantule, mais quelques sujets ne développent le branchage typique à partir du collet que lorsqu'ils arrivent à une phase plus avancée de végétation. Les analyses des données tirées de la progéniture ségréguée au point de vue de la semence bigarrée et claire et au point de vue du type de végétation montrent que ces caractères n'étaient pas associés dans la transmission héréditaire. L'examen des semences de 10,982 plants de mélilot ordinaire, cultivés en 1931, montre que 99.3 pour cent de la semence était incapable de germer à cause de l'imperméabilité de ses enveloppes. Les sélections individuelles de plants qui ont produit une proportion relativement élevée de semence naturellement perméable, ont été prises dans la variété de mélilot Arctique. La sélection dans les lignes consanguines des plants qui ont produit les plus grandes proportions de semence naturellement perméable, pendant deux générations successives, ont résulté en une forte augmentation dans la proportion de semences perméables produites et dans la proportion de plants produisant des semences perméables. La sélection continue des plants qui n'ont produit que des semences dures a résulté en une augmentation marquée dans la proportion de semences dures dans les lignées consanguines de la deuxième génération. Par le traitement des semences perméables avec l'acide osmique, on a établi que les régions perméables sont distribuées au hasard et qu'elles ne sont pas limitées à une région particulière de l'enveloppe de la semence. L'examen histologique des semences traitées à l'acide sulfurique a fait voir que la ligne claire est la région réelle d'imperméabilité dans les semences dures. Le traitement à l'acide sulfurique, a rendu perméables les semences apparemment dures, prises dans les lignées hautement perméables, mais seulement une petite proportion des semences dures provenant des lignées imperméables a été affectée par ce traitement. Les semences provenant de plants perméables, qui ne germaient que lentement dans les essais de laboratoire, peuvent être considérées comme pseudo-dures. Une forte proportion de ces semences germaient sans traitement lorsqu'elles étaient semées dans une terre humide. Les semences ayant des taches brunâtres ont été trouvées dans quelques-uns des plants provenant des lignées hautement perméables, et lorsque ces régions décolorées étaient traitées avec l'acide osmique, on a constaté qu'elle absorbaient aisément la solution. L'examen histologique de sections faites à travers les régions brunâtres a démontré que les cellules de l'enveloppe de la semence étaient quelque peu désorganisées. Les semences naturellement perméables dans ces lignées n'étaient pas limitées à celles qui possédaient les régions brunâtres. Beaucoup de semences d'apparence normale ont absorbé de l'eau et ont germé promptement sans être traitées.

BOOK REVIEWS

SNYDER, LAURENCE H.—The Principles of Heredity. D. C. Heath and Company, Boston. Price $3.00.

Dr. Snyder, Professor of Zoology in the Ohio State University, has prepared this volume for beginners courses in heredity. He has covered the development of this science during the first third of the present century.

Many of the publications on genetics and heredity now appearing are for advanced students and are often incomprehensible to the reader who may have had acquaintance with the subject in its earlier days and who now desires to "brush up" on the latest developments. Dr. Snyder's book will enable him to do this because it is written in simple terms and is exceedingly well illustrated. The terms of genetics used are printed in bold face type and followed by lucid explanations.

In subject matter the author has made use of a large mass of material selected from recent journal publications. A reference list accompanies each chapter. Considerable space is devoted to the genetics of domestic animals and to the inheritance of physical and mental traits in man. The chapter on eugenics, with the illustrations accompanying it, should convert anyone who has had any doubt as to the necessity of sterilization of criminals and defectives.

This book can be strongly recommended to those whose acquaintance with the subject of heredity is limited, and who desire to widen their knowledge of the fundamental principles of the subject.

H.L.T.

JONES, G. HOWARD.—The Earth Goddess. Longmans, Green and Company, Toronto. Price $3.50.

Mr. G. Howard Jones of the Ministry of Agriculture of Egypt, and formerly of the Department of Agriculture in Nigeria, has written a very interesting book on native farming on the West African Coast. This volume has been included in the Royal Empire Society Imperial Studies as Contribution No. 12 under the general editorship of Dr. A. P. Newton, Rhodes Professor of Imperial History at the University of London.

This book is more than a study of farming in West Africa. While there is much interesting descriptive material concerning the people and the agricultural methods, the general thesis is that the peasant proprietorship type of farming is to be preferred to the large estate system

under the control of European capitalists or "planters". In developing his thesis the author describes at considerable length the systems of government and education in West Africa.

In a chapter which he terms a "Digression on Scale in Agriculture" there is a very interesting analysis of the development of peasant proprietorship on the European Continent and the British Isles and its contrast with the large scale farming on the American Continent. Students of farm management and those generally interested in social problems in agriculture will find *The Earth Goddess* a very interesting contribution.

H.L.T.